# The structure, development and role of song in dippers.

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**This thesis is my own work, and has not been submitted in substantially the same form for the award of a higher degree elsewhere.**

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# Contents

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# Chapter 1

# General introduction

## 1.1 Introduction

A signal is defined as 'any act or structure which alters the behaviour of other organisms' (Maynard Smith & Harper 2003). Animals use olfactory, visual and acoustic signals to communicate state and condition of the signaller to a receiver. Individuals can also use a mix of these sensory channels to produce multimodal signals; these additional components can increase the success of the signal (Partan & Marler 2005). Biotic factors such as excessive ambient noise, can encourage visual signalling over vocalisations and vice versa with abiotic factors (Partan & Marler 2005). Evolution favours honest signalling, especially if the production of a signal is dependent on physical characteristics that are impossible or costly to fake (Krebs & Davies 2009).

The use of sound to communicate is beneficial when visual cues are unreliable, for example in poor light or dense vegetation; sound travels in all directions, over long distances and can rapidly transmit information efficiently (Catchpole & Slater 1995). In birds, song is one of the primary forms of communication, conveying a range of information between individuals but primarily functioning in mate attraction and territorial defence (Catchpole & Slater 1995). Individuals that are able to express superior traits are more likely to possess high quality genes and those in better condition are able to sustain song for longer (MacDougall-Shackleton & Spencer 2012; Nowicki et al. 2000; Ritschard & Brumm 2012); most birds therefore use song to advertise their quality (Catchpole & Slater 1995; Byers 2006). Song characteristics can

therefore provide the listener with an indication of the singer's current condition and developmental history together with key information such as mating status and territory size (Catchpole & Slater 1995; Nowicki et al. 2000).

### *1.1.1 Male song*

It is thought that the evolution of complex song in male birds is driven by sexual selection through female choice and competition between males; intersexual selection underpins the use of song for mate attraction whereas intrasexual competition is the major force behind the use of song for territorial defence (Catchpole 1980; Catchpole & Slater 1995). Occupying a territory can be a prerequisite for mate attraction and therefore crucial for reproductive success in many species (Armstrong 1963; Catchpole & Slater 1995). Within temperate regions, where breeding occurs seasonally, there is an increase in the incidence of male song with aggression and defence of territories (Catchpole & Slater 1995). Individual recognition is also an important feature of song in this context, as territory owners keep track of their neighbours in order to identify strangers, the latter often posing a greater threat in terms of territory ownership or extra-pair mating (Catchpole & Slater 1995; Stoddard 1996).

In many species, territory size and quality are important determinants of reproductive success and so are important factors in sexual selection (Dussord & Ritchison 2003). More experienced males that sing longer songs with larger repertoires obtain higher quality territories in the red-winged blackbird (*Agelaius phoeniceus*; Yasukawa et al. 1980), great reed warbler (*Acrocephalus arundinaceu*; Catchpole 1986) and pied flycatcher (*Ficedula hypoleuca;* Lampe & Espmark 2003). Moreover, males with better quality territories have been shown to attract females more quickly in the sedge warbler (*Acrocephalus schoenobaenus*; Buchanan & Catchpole 1997), song sparrow (*Melospiza melodi*; Hiebert et al. 1989), and Northern mockingbird (*Mimus polyglottos*; Howard 1974). In the sedge warbler, the amount of time spent in song flight, repertoire size and territory size were all found to have a significant negative correlation with pairing date (Birkhead et al. 1997; Buchanan & Catchpole 1997). Males that

arrived earlier to the breeding site and obtained better quality territories also sang songs with greater complexity, which further increased their likelihood of obtaining a mate sooner (Lampe & Espmark 2003). Thus, song can convey crucial information regarding a male's quality but also the quality of his territory, and females can make mate selection decisions accordingly.

### *1.1.2 Female song*

Although relatively understudied compared to males, females also sing in many species (Langmore 1998; Odom et al. 2014). Female song is often overlooked because the songs can be less frequent, limited to a specific period of the breeding cycle, or because a large number of the relevant species are monomorphic and female birds can be mistaken for males (Langmore 1998). However, recent years have seen an increase in the number of studies of female song which is now known to be widespread within the tropics and Australasia, and is considered by some to be ancestral (Odom et al. 2014). It has also been suggested that female song is a manifestation of the convergence of sex roles within the tropics, where there is a high prevalence of permanent monogamous pair-bonds and year-round territoriality (Morton 1996). In monomorphic, tropical or southern hemisphere species where the sex roles of a pair are reversed, females contribute to a large proportion of the singing within a pair (Illes 2015).

Duetting is one of the commonest forms of female song in the tropics; pairs which sing identical songs whilst duetting are thought to participate in cooperative defence against all intruders (Hall 2004; Langmore 1998; Logue 2005), whereas those which sing different songs are thought to be defending their own resources from same-sex individuals only (Hall 2004; Morton 1996). In some species, duetting is thought to maintain contact between a pair and assist in the coordination of breeding activities (McElroy & Ritchison 1996), enabling them to communicate motivational state to each other (Hovekamp 1996).

Females of some species sing solo songs and this usually relates to social and ecological conditions, such as the mating system or spacing between territories (Langmore 1998; Riebel et al. 2005). Solo female song has been suggested to serve a range of functions in different species,

including mate attraction, territorial defence, mate guarding, pair bond maintenance and the coordination of breeding activities (Langmore 1998). The Alpine accentor (*Prunella collaris*) is a polygynandrous species and because of the high levels of female-female competition, it is thought that female song advertises fertility to attract a mate (Langmore et al. 1996). The use of song for territorial defence in females has been found in European starlings (*Sturnus vulgaris*; Sandell & Smith 1997), yellow warblers (*Setophaga petechia*; Hobson & Sealy 1990) and Northern orioles (*Icterus galbula;* Beletsky 1982). Polygyny or extra-pair copulations can increase the amount of time males spend away from their nest and so in order to defend their own resources, females may use aggressive song to ward intruders away (Beletsky 1982b; Hobson & Sealy 1990; Langmore & Davies 1997; Sandell & Smith 1997). Conversely, female song is thought to have been lost in many species because it serves very little purpose where there are low-levels of intra-sexual aggression, for example in most non-migratory, monogamous species that reside in dispersed nest sites (Morton 1996).

## 1.2 Song development

Almost all songbirds learn their species-specific songs by copying tutors or models heard during the early phases of their life (Catchpole & Slater 1995; Nowicki et al. 1998). During the 'learning phase', birds first hear and memorise song and then reproduce these songs in a gradual process until they have matched their own vocal output to that of their tutor (Nowicki et al. 1998). This 'memorisation phase' is a short time period that typically occurs a week or two after hatching (Bolhuis & Gahr 2006; Catchpole & Slater 1995; Nowicki et al. 1998). It is followed by the 'motor phase', in which birds sing their own sub-song for a number of weeks until their stereotyped copies of song are crystallised (Armstrong 1963; Nowicki et al.

1998). This pattern is widespread across passerines, but not all learning in passerines is age-limited; in a small number of so-called 'open-ended learners', crystallisation does not occur and adults continue to alter their songs throughout their lives (Bolhuis & Gahr 2006; Müller et al. 2010).

Song learning and production in birds is controlled by the 'song system', which consists of a series of nuclei within the brain; the timing of song learning occurs parallel to the development of these brain structures (Nowicki et al. 1998). During the first few weeks of life, songbirds form auditory memories and rapidly develop brain regions such as the higher vocal centre nucleus (HVc) and robust nucleus of the arcopallium (RA; MacDonald et al. 2006; Spencer et al. 2003;). The HVc is a telencephic nucleus which is associated with the learning and production of song in birds (Spencer & MacDougall-Shackleton 2011). The RA is thought to be accountable for specifying the acoustic fine structure of individual notes or syllable types (Nowicki, W A Searcy, et al. 2002). In some species, HVc and RA sizes have been found to correlate with repertoire size (Nottebohm 1981; Spencer et al. 2003). These regions are separate when chicks hatch, but as nestlings develop the HVc sends neuronal projections to the RA; the connection of these nuclei also corresponds with an individual's first versions of sub-song (Nottebohm 1981; Spencer et al. 2003). Repertoire size links to the individual's ability to invest in brain tissue, and more energy invested enables better song learning in young birds (Nowicki, W A Searcy, et al. 2002). Brain tissue is costly to build and maintain, so only high quality males can invest and match the energetic demands required to produce a large song repertoire (Gorissen et al. 2005).

It has been observed in a range of species, including swamp sparrows (*Melospiza georgiana*) and song sparrows (*Melospiza melodia*), that individuals which have experienced higher levels of stress during development grow smaller HVc and RA, and consequently produce song with reduced complexity and show lower accuracy during song copying (MacDonald et al. 2006; Nowicki, W A Searcy, et al. 2002; Schmidt et al. 2013). Juvenile swamp sparrows were found to produce sub-song earlier; the adult song produced was therefore

a less accurate imitation of their tutor's song due to this shortening of the memorisation phase (Nowicki, W A Searcy, et al. 2002). The song centre in the brain develops simultaneously with the immune system and resources may therefore be interchanged depending on the priority for fitness (Kvarnemo 2011). Early life stressors may also have the capacity to affect an individual's willingness and ability to practise, which reduces auditory output and neural development (Spencer & MacDougall-Shackleton 2011).

## 1.3 The developmental stress hypothesis

Nowicki et al. (1998) developed the 'nutritional stress hypothesis', stating that periods of food deprivation or malnutrition experienced during the early stages of development would be represented in an individual's adult phenotype, including song. Genetic and external factors such as brood size, parasitism, disturbance, weather conditions and seasonality can also influence development; Buchanan et al (2003) therefore proposed that the hypothesis be broadened to the 'developmental stress hypothesis', encompassing any form of stressor to which individuals may be exposed in the earliest stages of life when they are particularly vulnerable (MacDougall-Shackleton & Spencer 2012).

The song control regions of the brain required for learning and production are very costly to build and maintain, so only those individuals which fare well during development or experience no stress have the required resources to invest heavily in such structures (Bonaparte et al. 2011; Spencer & MacDougall-Shackleton 2011; Zann & Cash 2008). Individuals that have been exposed to stress during early life often develop poorer quality song characteristics such as lower repertoire size, syllable number, song type, or complexity, or a weaker ability to copy a template or tutor's song (Kvarnemo 2011; Nowicki et al. 1998; Nowicki, W A Searcy, et al.

2002; Spencer & MacDougall-Shackleton 2011). Tarsus length, which provides a useful index of the quality of nutrition received in early life, is positively correlated with dawn repertoire size in male blue tits (*Cyanistes caeruleus*; Doutrelant et al. 2000). Similarly, in the great reed warbler (*Acrocephalus arundinaceus*), it was found that the length of the innermost primary feather predicted repertoire size in first year males (MacDougall-Shackleton & Spencer 2012; Nowicki et al. 2000). Most studies of the effect of developmental stress on song production have used captive birds; whilst effects detected in these studies can be meaningful, more field studies of wild birds are urgently needed to assess the extent to which these effects are biologically relevant (Byers & Kroodsma 2009).

### *1.3.1 Brood size*

Nutritional stress can be caused by sibling competition in the nest which is strongly influenced by brood size. In altricial species such as songbirds, all offspring depend on the finite amount of resources that parents provide and these may be unevenly distributed due to the size, sex and age of nestlings (Braasch et al. 2011). Older siblings dominate as they have the competitive advantage of hatching first (Nilsson & Svensson 1996); they gain energy and develop better locomotor skills faster and therefore starvation can be the most common form of mortality in smaller chicks (Nilsson & Svensson 1996). In larger broods, each nestling receives a smaller share of resources, leaving fewer available for processes such as song development. These higher levels of stress from sibling competition in larger brood sizes have been shown to reduce the ability of individuals to learn the syntactical structure of a tutor's song in zebra finches (*Taeniopygia guttata*; Holveck et al. 2008); decrease phrase syntactical complexity in Bengalese finches (*Lonchura striata domestica*; Soma et al. 2006); and decrease strophe length in blue tits (Dreiss et al. 2006).

### *1.3.2 Parental care*

Parental care comes in many forms: predator defence, nest-building, incubation and food provisioning (Greig-Smith 1982; Ketterson & Nolan 1994). Recording provisioning rates

is one of the most commonly used measures of parental care and one way of measuring nutritional stress in nestlings. Young birds of altricial species are completely dependent on provisioning by their parents when in the nest, and also for a brief period after fledging (Spencer & MacDougall-Shackleton 2011). When provisioning rates increase, this usually equates to an increase in offspring mass and therefore the probability of survival once fledged (Martin 1987; Schwagmeyer & Mock 2008). When parental care begins to cease, fledglings may not yet have fully developed the required skills to forage for themselves and these are the periods when fledglings are most susceptible to nutritional stress (Nowicki et al. 1998; Nowicki, W A Searcy, et al. 2002; Searcy et al. 2004). In a study of sedge warblers by Buchanan and Catchpole (2000), increased male provisioning rate was found to produce heavier nestlings. They also found a direct positive relationship between nestling mass and repertoire size, indicating that parental effort can have a major impact on a nestling's future fitness (Buchanan 2000).

### *1.3.3 Weather*

Weather can be a natural cause of stress in birds and adverse conditions can lead to unpredictable food shortages (Quillfeldt et al. 2007) and reduce the time available for foraging because individuals sometimes have to take shelter (King & Murphy 1985). Poor weather conditions have been shown to cause rises in circulating corticosterone levels in birds, thereby encouraging feeding behaviour (Møller & Swaddle 1997), but prolonged exposure to corticosterone can have detrimental long-term effects (Buchanan 2000). Individuals that cope better with unpredictable stress such as climatic factors will have a greater probability of survival (Krause et al. 2009).

### *1.3.4 Parasite load*

Parasite load has been found to affect song complexity in adult male canaries (*Serinus canaria*); individuals infected with malaria (*Plasmodium relictum*) developed reduced HVc and produced simpler songs (Spencer et al. 2005). This shows that developmental stress can interrupt the growth of brain nuclei, affecting trait expression later in life. Stressors have also

been observed to affect the immune system; European starlings that were exposed to endocrine disruptors were found to have larger HVc volumes, repertoire sizes and longer song bouts, but suffered from impaired immune function (Markman et al. 2008). In white-crowned sparrows and song sparrows, immigrant birds are immunologically disadvantaged and were more severely infected with blood-borne parasites (Macdougall-Shackleton et al. 2002; Stewart & MacDougall-Shackleton 2008). These males are thought to lack experience in the local parasite fauna and this results in lower reproductive success, as females prefer males that sing songs with the local song dialect (Macdougall-Shackleton et al. 2002; Stewart & MacDougall-Shackleton 2008).

### *1.3.5 Pollution*

Gorissen et al. (2005) found that singing behaviour in great tits (*Parus major*) was affected by environmental stress caused by pollution. Heavy metals are a frequent by-product of agriculture and industry and they can enter the food chain through air, water, soil and biota; bioaccumulation of these pollutants can occur with concentrations increasing further up the food chain (Burger 1993). Males closer to the source of pollution were found to sing up to 35% less and had a significantly smaller repertoire size (30%) than those further away from the site (Gorissen et al. 2005). Pollution can decrease resource availability which in turn causes an increase in time costs because males need to forage for longer and so have less time available for singing (Gorissen et al. 2005).

### *1.3.6 Adaptations to stress*

Individuals best able to endure stressors during early life should produce the most attractive songs as adults (MacDonald et al. 2006). During periods of stress, the stress hormone corticosterone is released and this has adaptive uses such as the mobilisation of stored energy (Fokidis et al. 2012); however, prolonged exposure to corticosterone can lead to damaging effects on neurological development and immunosupression (Buchanan 2000). This can result in an individual with a reduced ability to produce a complex song (Byers 2006; Darolová et al.

2012; Kagawa & Soma 2013; Mountjoy & Lemon 1996), thereby affecting its lifetime reproductive success (Nowicki et al. 1998).

Birds use a variety of mechanisms to adapt to stress as young: they can delay maturation and fledging to avoid stressors affecting their adult phenotypes; use compensatory growth by developing faster than usual; or invest resources in characteristics that are more important for current condition and survival (Searcy et al. 2004; Spencer & MacDougall-Shackleton 2011). Compensatory growth is often costly and can have consequences for adult phenotypes (Metcalfe & Monaghan 2001). In a study of the zebra finch, metabolic rates, cognitive abilities, life history stages and exploratory behaviour were all found to be affected by compensatory growth (Krause & Naguib 2011). Given the above evidence, it is likely that compensatory growth strategies will have an effect on song development and production but this requires further study.

Most birds are close-ended learners which means that after crystallisation in their first year, there are no additions to the song repertoire (Catchpole & Slater 1995). The adult song of these close-ended learners is a clear reflection of how a bird managed during exposure to stress in early life and indicates the outcome of his learning during postnatal development (Nowicki et al. 1998). For open-ended learners, song can provide a useful index of their present condition in light of environmental stressors, and those that have more resources available will invest more time and energy in song production (MacDougall-Shackleton & Spencer 2012).

## 1.4 Aims and thesis outline

The aim of this project was to investigate the structure and function of male and female song in dippers, and whether developmental stress plays a role in its development (Figure 1). I begin by describing the differences between male and female song in terms of complexity, frequency and temporal characteristics, and the seasonality with which each sex sings (Chapter 3). Male song structure is also compared between the different contexts in which singing was recorded. I continue to investigate the use of song in both sexes in the context of territorial defence, simulating unfamiliar intruders in a playback experiment (Chapter 4). I then investigate how variation in early life conditions may influence the characteristics of song produced in adulthood (Chapter 5) and the extent to which these song characteristics predict reproductive success in males (Chapter 6). In a second experiment, I use dietary manipulation to investigate variation in nestling growth and the long-term impacts on the development of adult song. Finally, I discuss all of my findings in the context of previous studies, with suggestions for future work (Chapter 8).

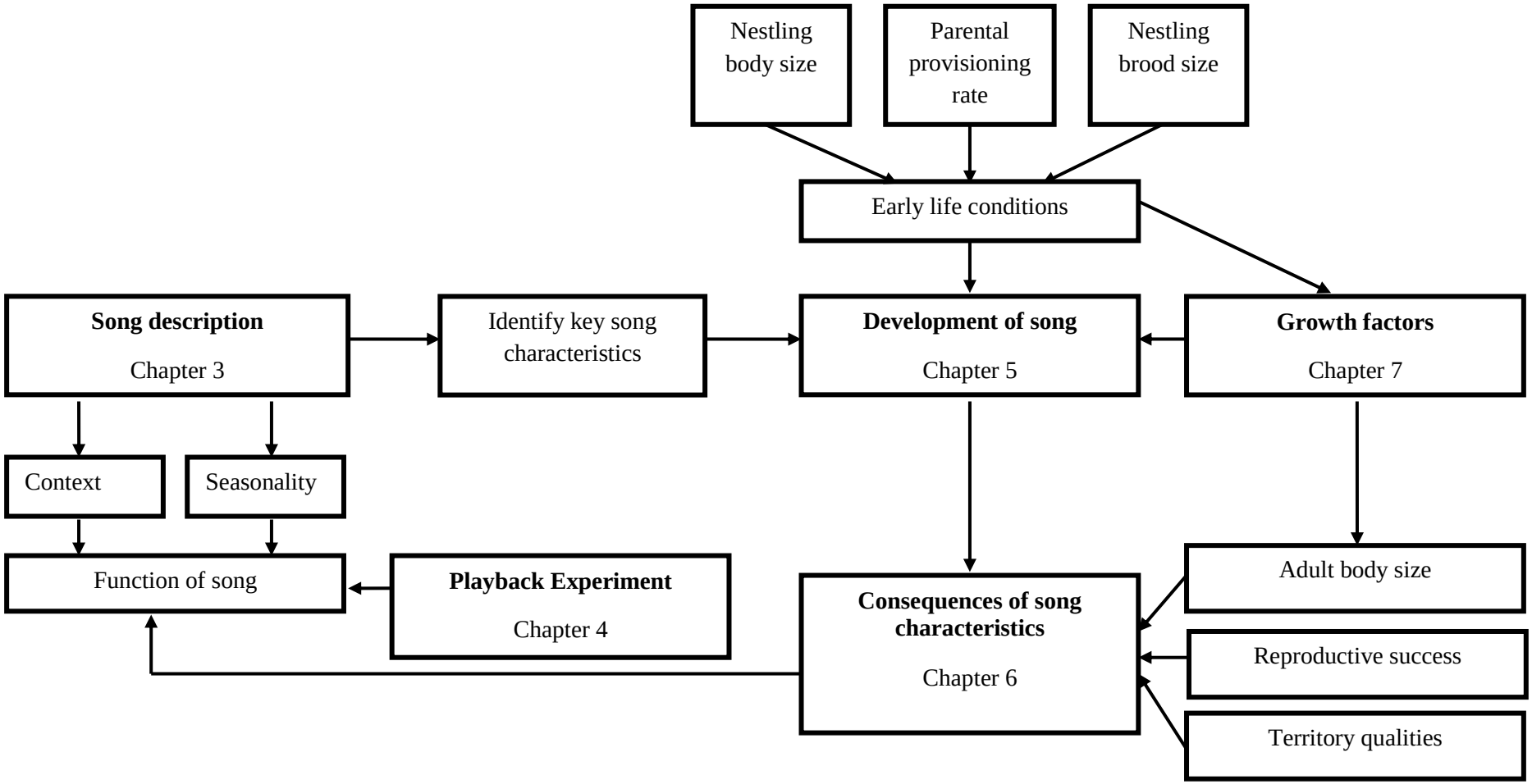


Figure 1. A conceptual model of the relationship between the different elements of this project, the flow of arrows represents variables that contribute to each element; the chapter for each element of the model is also included.

## 1.5 The dipper (*Cinclus cinclus*)

### *1.5.1 Introduction*

The *Cinclidae* are a unique family of aquatic passerines found on fast flowing rivers and streams (D'Amico & Hémery 2007; O'Halloran et al. 2003). They are widely recognised as an avian bio-indicator for freshwater habitats because they are obligate predators of benthic macro-invertebrates and small fish, and therefore sensitive to environmental quality (D'Amico & Hémery 2007; Henny et al. 2005; Morrissey et al. 2010b; Morrissey et al. 2013; Vickery 1992). There are five species of dipper: white-throated (*Cinclus cinclus*), American (*C. mexicanus*), brown (*C. pallasii*), white-capped (*C. leucocephalus*) and rufous-throated (*C. schulzi).* These species are distributed across the world in freshwater habitats: from North Western to Central America (American), Europe, the Middle East, Central Asia and the Indian Subcontinent (white-throated), Southern and Central Asia (brown), Bolivia, Colombia, Ecuador, Peru and Venezuela (white-capped) and Bolivia and Argentina (rufous-throated) (Ormerod & Tyler 2005; Willson & Hocker 2008). Hereafter, any use of the words 'dipper' or 'dippers' will refer to the white-throated dipper unless otherwise stated.

Dippers are rotund, chestnut brown birds with a white plumage on their throat and chest and measure 16-20cm in length. They are sexually monomorphic in plumage but males are larger, weighing 65-75g with wing lengths over 92mm long (Tyler & Ormerod 1994); females weigh 55-60g and rarely have wing lengths over 92mm (Tyler & Ormerod 1994). Dippers live on rivers and streams, building their nests close to fast flowing water, and pairs defend linear territories during the breeding season (Ormerod & Tyler 1991; Nybø et al. 1997) and often over winter (Crowther 2016; Tyler & Ormerod 1994). They forage by diving underwater and manoeuvre themselves using their wings and feet (Tyler & Ormerod 1994), or by wading along river margins and foraging between stones (Eguchi 1990; Tyler & Ormerod 1994). On average, dippers will spend 45-55% of daylight hours foraging with the remaining time spent resting.

True to their name, dippers dip up and down, a behaviour usually reserved for territorial, courtship or alarm purposes (Tyler & Ormerod 1994). This dipping behaviour is commonly seen in other riverine species such as wagtails (*Motacilla sp.*) and is thought to make the birds look less conspicuous against the ever-moving background of their environment (Tyler & Ormerod 1994). The white plumage on the white-throated and white-capped dippers is also thought to break up the outline of the body against the water, contributing to reduced detection and predation (Ormerod & Tyler 2005). Frequent blinking is another behaviour dippers exhibit, and this is thought to be part of their courtship or threat displays; it is made particularly obvious by their white eyelids (Tyler & Ormerod 1994).

### *1.5.2 Adaptations to aquatic life*

Dippers are highly adapted to their aquatic lifestyle as their size, shape and strength all contribute to their ability to live on and under the water (Ormerod & Tyler 2005). All dipper species have long legs with strong toes to grip onto the substrate below the water; they have relatively short wings for a bird their size; and powerful musculature which gives them greater manoeuvrability underwater (Tyler & Ormerod 1994). Dives can last up to 23 seconds long but are usually around 3.2-3.6 seconds with an average of 5.1 dives per minute (Tyler & Ormerod 1994). Dippers have extremely dense plumage and a large preen gland for waterproofing their feathers, and a sphincter muscle within their iris that can change the curvature of the lens, giving them greater power of accommodation and better vision underwater (Tyler & Ormerod 1994). They also have a higher level of haemoglobin in their blood compared to other passerines and can decrease their heart rate whilst diving, which enables them to increase their oxygen storage and dive for longer (Tyler & Ormerod 1994).

### *1.5.3 Habitat and territory quality*

Dippers are socially monogamous, and extrapair paternity is reportedly low in this species (Øigarden et al. 2010). Territories can vary in length from 300-2500m and are usually occupied by a monogamous pair. Better quality rivers have been shown to have a greater

abundance of breeding pairs (Tyler & Ormerod 1994). Characteristics such as prey abundance, water quality, riffle area, stream gradient and availability of suitable nest sites are all important aspects of territory quality (Ormerod & Tyler 1989; Tyler & Ormerod 1994). Nests are usually placed on rocky banks, cliffs, within fallen trees or roots, with the most popular locations being man-made structures such as culverts, bridges or nest boxes (Tyler & Ormerod 1994). Nest and fledging success of the American dipper have been positively correlated with an index of nest site quality, so site selection is important for an individual's fitness (Price & Bock 1983); the height and location of the nest also determine the risk of flooding, a major source of nest failure in these birds (Tyler & Ormerod 1994). Well-oxygenated water with stony beds provides the perfect habitat for aquatic invertebrate prey, therefore patches of riffles that provide this aeration are important and territories with larger areas of riffles are considered to be of greater quality (Ormerod et al. 1986; Ormerod & Tyler 2005). Studies of American dipper populations have found that territory quality and food abundance are key for fledging success and recruitment into the breeding population; this does not always equate to territory length which may sometimes be larger where good feeding sites are more scattered (Feck & Hall 2004; Price & Bock 1983).

### *1.5.4 Breeding ecology*

Most dippers remain on their breeding territories during the winter but in locations where rivers freeze over or conditions become unfavourable, birds will undertake altitudinal migration downstream or to the coast for other foraging opportunities (Tyler & Ormerod 1994). This migration can cause large aggregations of dippers and during the autumn they roost communally under bridges or in stone crevices (Tyler & Ormerod 1994). Territorial behaviour starts between September and November after the moult period when individuals begin to defend their winter territories (Tyler & Ormerod 1994). Dominant individuals benefit by successfully gaining a high quality territory, granting access to high quality feeding sites and therefore the opportunity to attract better quality mates (Bryant & Newton 1994). Many

breeding pairs remain together throughout the winter and defend their territory as a pair, but single birds begin to pair up in later winter (Crowther, personal observations).

Pairs begin to build nests from the end of January (Tyler & Ormerod 1994) and do this by repairing an old nest from a previous season or building a new one; whichever the case, the lining for each nesting attempt is new each time (Nilsson et al. 2011). Dipper nests can take up to 28 days to construct; they are dome shaped and approximately 13-22 cm in diameter, with an external structure of moss, plant fibres and sometimes lichen, and a grass cup usually lined with leaves (Tyler & Ormerod 1994). Bigamy can occur but usually only opportunistically; in these instances, males defend longer stretches of river with a boundary line in the middle, and the two females have a nest each in either half (Tyler & Ormerod 1994).

Dippers are early breeders compared to other bird species with laying generally starting in March but later on higher ground; pairs can have two clutches within a season if they begin the first in March or early April (Tyler & Ormerod 1985). One egg is laid per day with clutches containing 4-6 eggs on average (Tyler & Ormerod 1994). Incubation begins once all eggs are laid and lasts 16-18 days, with chicks fledging after approximately 20-22 days (Tyler & Ormerod 1994). Breeding success improves with experience; pairs in Scotland had greater success when both parents were adults than when at least one bird was a first-year (Wilson 1996). Adults remain with their offspring for up to two weeks after fledging to assist in feeding which increases their chances of survival whilst they develop their foraging skills (Tyler & Ormerod 1994). Causes of nest failure include: desertion, flooding, nests falling into the water, human disturbance and predation by mammals or corvids (Smiddy et al. 1995; Tyler et al. 1990; Willson & Hocker 2008).

### *1.5.5 Diet and foraging behaviour*

Dippers feed almost exclusively on aquatic macro-invertebrates and small fish (Ormerod & Tyler 1991). Mayfly (Ephemeroptera), stonefly (Plecoptera) and caddis fly (Trichoptera) larvae make up a large part of the diet (Ormerod & Tyler 1991). Smaller prey

items such as Baetid mayfly larvae are fed to newly hatched offspring due to their small gape and gut sizes; as the chicks grow, their diet switches mostly to caddis fly larvae that supply a higher rate of energy per delivery due to their size (Ormerod & Tyler 1991; Taylor & O'Halloran 2001).

When foraging for themselves, juveniles specialise on small, stationary prey such as fly larvae (Diptera; Yoerg 1998). They do not dive because they lack the coordination and strength required, sticking to slow flowing, shallow patches of water from which to forage (Yoerg 1994). Mastering the foraging skills of an adult takes time and practise, and capture rates by juveniles are often low (Yoerg 1994). Juvenile mortality is high at this time due to increased predation and high starvation risk (Yoerg 1994). Young which feed on smaller prey items and rely on a more readily available food source have been shown to gain independence faster and disperse sooner; individuals that leave the natal territory first benefit from having first access to territories close by (Yoerg 1998).

During periods of spate, increased depth, velocity and turbidity can prevent dippers from foraging underwater and they are often forced to forage terrestrially (Taylor & O'Halloran 2001). Prey type switching has also been observed during periods of spate when individuals of an Irish population were seen to eat fewer caddis fly larvae and more mayfly nymphs (Taylor & O'Halloran 2001). Caddis fly larvae are less available at these times and the high water levels make them less energetically profitable to dive for (Taylor & O'Halloran 2001). Other dietary shifts have been observed in females, for example supplementing their diets with fish (Cottidae) to increase the calcium available prior to laying (Ormerod & Tyler 1991; Willson & Hocker 2008).

### *1.5.6 An indicator of environmental quality*

Dippers are widely recognised as important indicators of water quality (Sorace et al. 2002). They are especially suited for bio-monitoring because they provide a useful index of local conditions, feeding exclusively on aquatic organisms which are sensitive to pollution and

being year-round residents in discrete territories (Henny et al. 2005; Morrissey et al. 2004; Nybø et al. 1996; Ormerod et al. 1988). Acidification of surface waters can be caused by acidic precipitation, conifer afforestation or man-made releases of sulphur and nitrogen oxides (Ormerod et al. 1988; Vickery 1992; Wilson 1996). Dipper population densities in Wales and Scotland were found to be lower where the water was more acidic, or had lower mineral content (Ormerod et al. 1985; Ormerod et al. 1986; Vickery 1992), and breeding success is also affected negatively by acidity (Logie et al. 1996; Ormerod et al. 1985; Ormerod et al. 1986; Sorace et al. 2002). Acid deposition can cause a calcium shortage which creates eggshell defects, and food stress (Logie et al. 1996; Morrissey et al. 2010b; Ormerod et al. 1985; Ormerod & Tyler 1989). A decrease in invertebrate prey means there may be a calcium shortage for females laying eggs (Wilson 1996). Eggs laid in acidified areas of Norway were 7% lighter and 6.1% thinner compared to those in non-acidified areas; the eggs also had a thinner palisade layer due to an impaired crystallisation of calcium carbonate (Nybø et al. 1997). A decrease in eggshell thickness can increase the diffusion capacity of the egg causing large amounts of water loss and desiccation of the embryos, dramatically reducing reproductive success (Nybø et al. 1997). Invertebrates are pollution intolerant (Feck & Hall 2004; Morrissey et al. 2010a; Sorace et al. 2002) and changes to water quality not only affects the food web, but also affects a dipper's ability to meet its own energetic requirements and that of its offspring, causing a decrease in condition and affecting future fitness (Logie et al. 1996).

Dipper eggs can also be a good source of information regarding water chemistry and contamination; nutrients deposited into eggs are from recent dietary uptake as passerines are unable to store sufficient nutrients on a daily basis in order to lay consecutively (Morrissey et al. 2004; Morrissey et al. 2010). This also enables the presence of contaminants to be linked to specific locations; for instance concentrations of polychlorinated biphenyls (PCBs) in eggs and local environments are frequently correlated (Nybø et al. 1997; Ormerod et al. 2000). Animals at higher trophic levels suffer from bioaccumulation and magnification of persistent organic

pollutants such as dichlorodiphenyltrichloroethane (DDT) and PCBs (Kallenborn et al. 1998; Tyler & Ormerod 1992).

### *1.5.7 Song*

All five species have loud, musical songs and both sexes are thought to sing throughout the year except when feeding young and moulting (Tyler & Ormerod 1994). In white-throated dippers, a peak in singing takes place over September and October when they are establishing winter territories and again from January to March to reinforce breeding territories (Ormerod & Tyler 2005). They sing from conspicuous perches along their territory (personal observations) and pairs will call and chatter to each other and alert one another to predators or other disturbances with loud alarm calls (Ormerod & Tyler 2005). Male song is thought to be used to attract a mate, communicate with partners and defend territories (Tyler & Ormerod 1994), whereas females have been suggested to sing in order to communicate with their partner (Tyler & Ormerod 1994). However, no formal analysis or experimentation has yet been performed on dipper song.

# Chapter 2

# General methods

## 2.1 Study site and population

### *2.1.1 Study site*

All of the fieldwork for this project was carried out in the River Lune catchment within a four-mile radius of Sedbergh, Cumbria, UK (54°323'N, 2°528'W) between 2014 and 2016 (Figure 1). Within the area four rivers converge: the Dee, the Rawthey, the Clough and the Lune itself. Each river has a number of streams and becks flowing into it from various hills within the study site, and together these watercourses provide numerous territories within which dippers can breed. All of the rivers and most of the streams were exploited by dippers, with only large stretches of deep, slow flowing water, or areas with no conceivable nesting sites remaining empty. The size and productivity of this population were monitored from 1958 to 1966 and again from 2003 to 2012, during which time traditional nesting sites were identified and various key life history events recorded. Then, in 2013, all birds in the population were fitted with unique combinations of colour rings (see below) and an individual-based monitoring programme has been carried out even since; a pedigree has been established from observational and ringing data. Between 2014 and 2016, the population size ranged from 45-47 breeding pairs.

### *2.1.2 Age, sex and body size*

Each year, all unringed adults are caught in mist nets or in hand nets placed over the nest; nestlings are ringed when they are nine days old. Every individual is given a unique

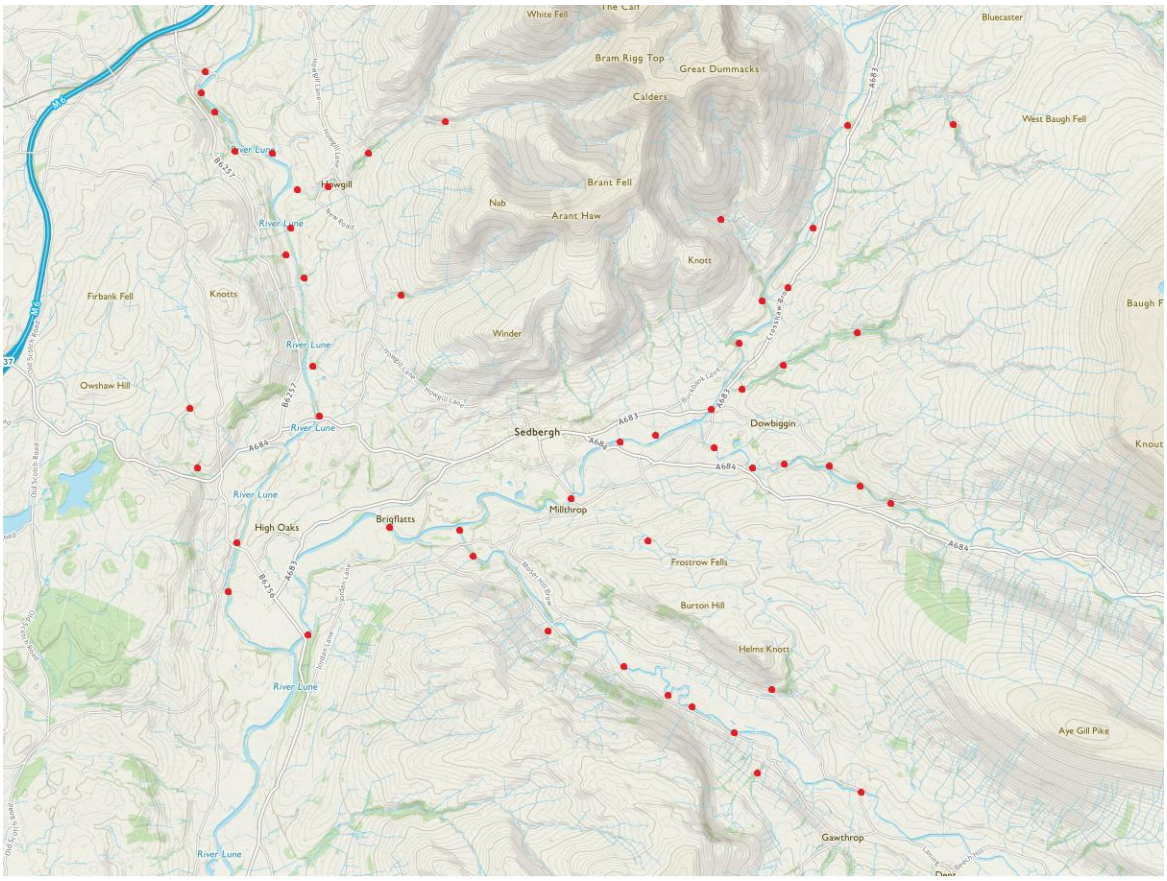


**Figure 1.** Ordinance Survey map of the dipper study area surrounding the village of Sedbergh (Cumbria), with 52 nest sites indicated in red.

combination of three plastic colour rings and a standard British Trust for Ornithology metal ring. There is no evidence that ringing has an effect on nestling mortality or nest desertion by the adults (females are not caught on the nest during the first week of incubation when the risk of desertion is probably higher (Kania 1992). The following morphological measurements are taken at capture from all full-grown individuals: (1) wing length (± 0.5 mm) taken from the carpal joint to the end of the longest primary on a closed wing using a capped wing rule; (2) tarsus length (± 0.1 mm) or 'maximum tarsus' is the length of the tarsometatarsus, measured using dial callipers; and (3) body mass (± 0.1 g) using electronic scales. Individuals are also aged and sexed at capture according to their wing moult and wing length, respectively (Andersson & Wester 1971; Svensson 1992). For most individuals, sexing could be confirmed from observations of reproductive behaviour (incubation is carried out by the female only; Tyler & Ormerod 1994) and in no cases was there a discrepancy between the two methods.

### *2.1.3 Breeding ecology*

All nests are found by following birds carrying nest material or by regularly inspecting traditional sites, and once located they are closely monitored until fledging or failure. As all stretches of river or stream are regularly monitored, it is very unlikely that any nests are missed in any given year. One or two nests were inaccessible in some years, but if these nests were successful then the fledglings were caught in mist nets soon after. Once a pair has been observed lining their nest with leaves, the nest is visited every 3-4 days thereafter in order to determine lay date (i.e. the date on which the first egg of the clutch was laid). Dippers lay one egg per day and rarely start incubating until the final egg has been laid (Tyler & Ormerod 1994), so lay date can be back-calculated once an incomplete clutch of cold eggs has been recorded. Clutch size is measured approximately one week after incubation has begun by counting the total number of eggs present. After a typical incubation period of 16 days (Tyler & Ormerod 1994), nests are checked daily for hatching; the date of hatching is occasionally missed by up to two days but the nestlings can then be aged from their appearance. Nestlings are removed from the nest when nine days old (hereafter known as 'day 9'); they are then ringed

and measured in the same way as adults. Nests are then checked at least once per week until fledging is due; from day 21 of the nestling period, daily checks are made to confirm fledging and to inspect the nest for any dead nestlings. For all successful nests, the number of chicks fledged was taken to be the brood size at ringing minus the number of dead nestlings found, as mortality after day 9 is rare.

### *2.1.4 Provisioning rate*

Offspring provisioning rates were measured during 1h nest observations conducted at least 15 m from the nest and using a portable hide if no natural hiding place was available. In a small number of cases, manual observations were not possible (e.g. if the nest was inside a culvert) and so recordings were made using a Panasonic HC-V160 video camera placed at least 5 m from the nest. For both manual observations and video recordings, the 1 h observation period did not begin until after the first time an adult visited the nest. This minimised the effect of observer or camera presence on provisioning rates, although very few birds ever seemed aware of the observer or camera and in the vast majority of cases offspring provisioning took place within a few minutes of the observer hiding or leaving the camera in place. Provisioning rates were measured by simply counting the total number of feeds made by the two parents during the hour. For each nest, observations were conducted between dawn and noon once per week during the 20-22 day nestling period, on day 2 (= 'Week 1'), day 10/11 (= 'Week 2') and day 17/18 (= 'Week 3').

## 2.2 Song analyses

### *2.2.1 Song recording*

All of the recordings of dipper song in this study were made in the field between January 2014 and June 2016. Recording sessions were conducted in daylight hours between 05:00 and 17:00. All recordings were made in approximately similar conditions, as high levels of wind can affect the quality of the sound recorded and torrential rain had the potential to damage the equipment as well as compromising the observer's vision. Songs were recorded from distances of 20-30 m using a Sennheiser ME66-K6 shotgun microphone, low frequency cut off switch turned off, with a Rycote Softie windshield and a standard pistol grip connected to a Marantz PMD661 MKII solid state recorder. Spectrograms were produced using Avisoft SASlab Pro, version 5.2.08 (Specht 1993), with a 512-point fast Fourier transform length and Hamming window function, 75% frame size, a 87.5% window overlap 86 Hz, frequency resolution and 1.4512 ms time resolution. A range of frequency and temporal characteristics were also measured using Luscinia version 2.02.10.15; spectrograms were produced using a fast Fourier transformation with a Gaussian analysis window, 80% spectrogram overlap, 10 kHz max frequency, 5 ms frame duration, 1 ms time step and 2 dB noise removal (Lachlan, 2007). All measurements were based on peak frequency, and taken by syllable.

### *2.2.2 Acoustic structure*

All measures of acoustic structure were obtained using ten songs from each of 34 males and 11 females. Songs, by definition are longer and more complex than calls, and usually are differentiated by function; songs can be associated with courtship and mating, whereas calls function as alarms or for cohesion within a group (Ehrlich et al. 2008). Songs with a low signal to noise ratio were excluded, but songs were otherwise selected at random from individuals for which more than ten songs were available (10 being the lowest number of high quality songs

available for any individual in the sample). In order to measure the size of each individual's repertoire, visual and auditory inspections of spectrograms were first used to identify elements (the smallest continuous tracing on a spectrogram), which were then grouped into syllables where appropriate (multiple elements made up a syllable, the pause between elements was less than 0.1 s and these elements were never given in isolation; Figure 2). Each unique syllable was catalogued by a method known as enumeration, for which the number of unique signal types present within a sample are counted (Botero et al. 2008). Syllable repertoire size (hereafter, simply 'repertoire size') was then calculated as the number of unique syllable types found across 10 songs for each individual. For species with large repertoires, Botero et al. (2008) found that simple enumeration was the best method when samples of songs are incomplete, something that is usually unavoidable when working with wild birds. Standardising sample size is recommended, in species with potentially large repertoires when estimating repertoire size (Botero et al. 2008). It is unlikely that listeners are counting entire repertoires as listening to an individual's full repertoire would be extremely time costly (Botero et al. 2008), and other cues such as syllable detection probability are likely to be in action (Garamszegi et al. 2005).

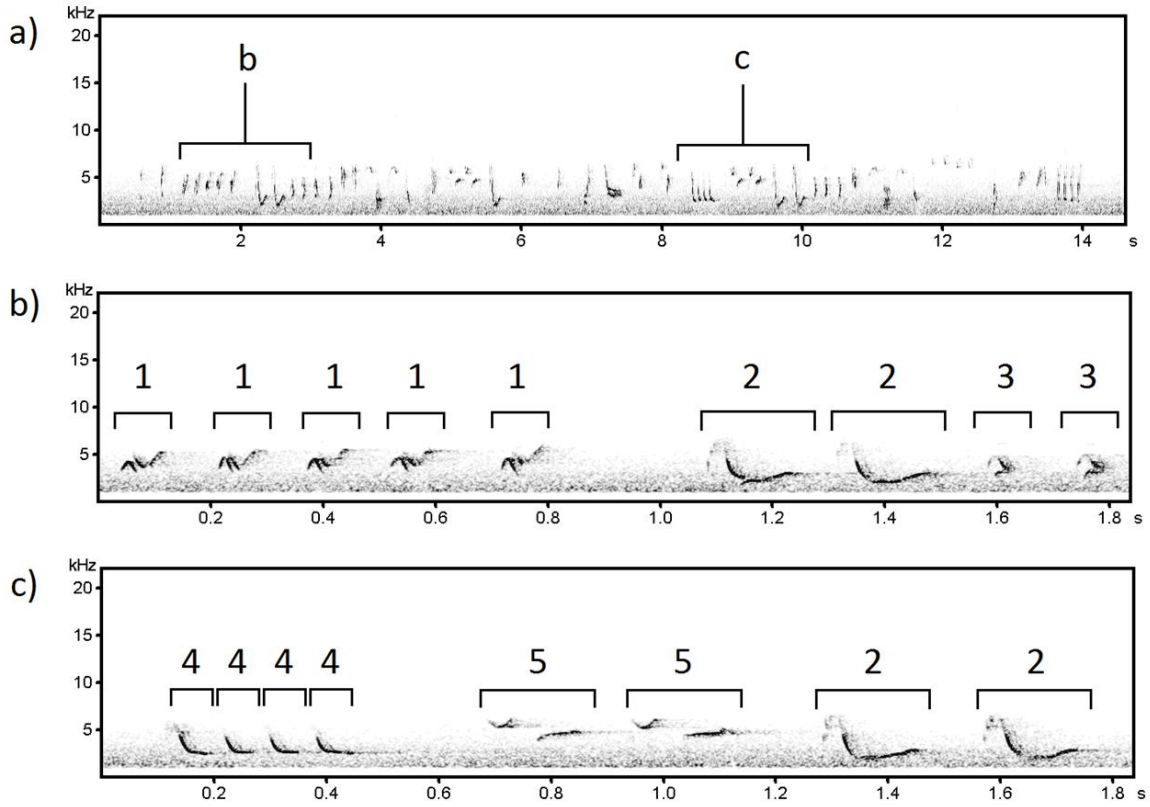


**Figure 2.** Spectrograms of male dipper song: a) full song; followed by sections of that song b) and c), which represent the resolution at which songs were analysed (Avisoft settings as stated in methods). Syllables are numbered, 1 and 5 showing how elements are grouped together to form syllables. Syllables can be repeated straight away (e.g. 1) or appear later in the same song (e.g. 2).

## 2.3 Territory quality

### *2.3.1 Kick sampling*

Primary feeding locations for each pair were identified during nest watches i.e. riffles within each territory that the adults were seen visiting most frequently to forage. Three minute kick samples were collected from these locations during the final week of the nestling period using standard methodology (Freshwater Biological Association 2017). The observer stood in the riffle to be sampled, placing a standard 1 mm mesh pond net on their downstream side. The substrate was then disturbed by kicking and the debris lifted was collected in the hand net using a sweeping motion. Samples were deposited into clear sealable polythene bags and preserved on site in 50% methanol and 50% water. Prior to analysis, all samples were strained through 0.5 mm and 4 mm stacked sieves. All specimens were then counted, identified to family level and weighed to give 'invertebrate mass' using an electronic balance (±0.01 g). Information on which families each sample contained was then used to calculate a Biological Monitoring Working Party (BMWP) water quality score. This index was designed to detect organic pollution by assigning families a score between 1 and 10 based on their sensitivity to pollution levels (Wenn 2008). A site's score is found by adding together all scores of the families present in the sample (Armitage et al. 1983). The BMWP score is then divided by the number of scoring taxa present in the sample to give Average Score per Taxa (ASPT). A larger ASPT value is considered indicative of a cleaner site (Wenn 2008), and gives a finer scale of water quality than BMWP. The altitude of each sampling (i.e. feeding) site was recorded to the nearest metre using a Garmin GPS MAP64.

### *2.3.2 Water chemistry*

In-situ chemical analyses were also carried out at primary feeding sites to assess water quality using meters placed directly into the river or stream; pH and electrical conductivity (in

µS) were measured using Hanna HI-98127 and HI-981311 probes, respectively; dissolved oxygen content (in ppm) was measured using a YSI Pro20 dissolved oxygen meter. Previous studies have shown pH to have a negative effect on dipper laying dates, egg shell thickness, egg mass, clutch and brood sizes, nestling mass, growth and survival, provisioning rates, incidence of second clutches and adult mass (Ormerod et al. 1988; Ormerod et al. 1991; Vickery 1992). At least some of these effects arise from the impact water acidity has on the availability of food, particularly calcium rich prey (Ormerod et al. 1988; Tyler & Ormerod 1992); therefore, pH provides another index of territory quality. Electrical conductivity (EC) is also known as molar conductivity and measured in Siemens (S), and can be affected by pollutants and inorganic solids dissolved in the water; lower levels indicate the sample is cleaner and therefore the water is of higher quality. Higher levels of dissolved oxygen content (DOC) also indicate better water quality as there is more oxygen readily available for organisms to utilise; cases of eutrophication decrease these levels (DEFRA 2010).

# Chapter 3

# Structure and function of male and female song

## 3.1 Abstract

Female song is more widespread than previously thought but remains poorly studied outside the tropics. Relatively few studies have compared the structure and function of female song with that of conspecific males, especially in non-duetting species. Here I investigate the acoustic structure, behavioural context and seasonal production of song in male and female dippers (*Cinclus cinclus*). The dipper is a highly territorial and socially monogamous passerine; although it has been reported that both sexes possess a highly complex song, very little else is known except they rarely sing during the late summer months whilst moulting (Tyler & Ormerod 1994). The songs of individually marked birds from a wild UK breeding population were recorded over a three-year period. The songs from females had similar acoustic characteristics to males but, on average, contained fewer syllables per second and higher levels of versatility, a measure of structural complexity. Unpaired males recorded early in the breeding season sang more complex songs at a higher rate than males that were paired up and nest-building or whose breeding attempts were underway, thus providing strong evidence that male song is primarily used for mate attraction. By contrast, females sang most often during aggressive encounters with birds from outside the territory. Furthermore, males sang throughout the breeding season, whereas females rarely sang after laying had begun. These results clearly show that song production and structure in dippers are highly dependent on the breeding stage and differ between the sexes; whereas male song serves to attract females and defend territories,

female song appears to primarily function in mate or territory defence. The wider implications of these findings are discussed, particularly regarding the possible function of female song in helping males and females to coordinate their breeding activities and thus maximise their reproductive success.

## 3.2 Introduction

The two main functions of song in male birds are mate attraction and territorial defence (Catchpole & Slater 1995). Song is thought to be an honest signal of male quality as it is energetically costly to produce (Catchpole & Slater 1995; Gil & Gahr 2002; Nowicki et al. 1998; Searcy & Yasukawa 1996). In addition to the energetic costs incurred, time spent singing represents time taken away from other activities like foraging (Oberweger & Goller 2001) and song can increase an individual's conspicuousness and therefore predation risk (Gil & Gahr 2002). Song complexity is a sexually selected trait in many species (Catchpole 1987; Macdougall-Shackleton 1997; Searcy & Andersson 1986), and characteristics such as repertoire size and song length have been shown to correlate with the size of song areas within the brain (Garamszegi & Eens 2004). An increase in size of regions of the brain such as the higher vocal centre (HVc) is associated with an increase in repertoire size and therefore the ability to learn a larger quantity of song. Stress experienced during brain development may have a lasting effect on song learning as only those individuals in better condition can afford the costs associated with brain size (Devoogd et al. 1993; Nowicki, W. A. Searcy, et al. 2002). Singing therefore allows males to relay their fitness, and by extension their territory quality or ability to provide a higher standard of parental care (Buchanan & Catchpole 2000; Kroodsma & Byers 1991)

alongside more fundamental information such as species and individual identity (Kroodsma & Byers 1991).

Repertoire size is a measure of either the number of unique songs or syllable types available to an individual (Gorissen et al. 2005; Hiebert et al. 1989; Potvin & Clegg 2015). In many species, males and females are sensitive to the variability within song, and repertoire size is thought to function in intrasexual selection, mate choice and reproductive stimulation (Hiebert et al. 1989). Song has been found to correlate with a number of individual and life history traits including condition, territory size and tenure, parental effort, reproductive success and the timing of migration (Buchanan & Catchpole 2000; Hesler et al. 2012; Hiebert et al. 1989; Kipper et al. 2006). Furthermore, repertoire use can change with context, season or age (Garamszegi et al. 2005). For example, comparisons of song in male bobolinks (*Dolichonyx oryzivorus*) showed that males sang shorter, less complex and more repetitive songs when they were presented with conspecific males than when presented with a female (Ammer & Capp 1999); male field sparrows (*Spizella pusilla*) sang songs of greater complexity during aggressive interactions (Nelson & Croner 1991). These studies provide evidence that songs within different behavioural contexts can reserve different structural features, often giving a clue to their function (Catchpole 1983; Järvi et al. 1980; Nagle & Couroux 2000; Payne 1979). However, further research on intraspecific variation in song structure across different behavioural contexts is needed.

Female song was once thought to be rare but recent work has shown that it is widespread across taxa, especially in the tropics and Australasia (Illes 2015; Odom et al. 2014). Most research has focussed on duetting species (Hall & Peters 2008; Mennill & Rogers 2006; Rogers et al. 2006; Topp & Mennill 2008) but far less is known about the function of solo female song in other birds (Langmore 1998). In some cases there is evidence for a role in territory defence, including monogamous species with year-round territoriality (Brunton & Li 2006; Price et al. 2008) and polygynous species such as red-winged blackbirds (*Agelaius phoeniceus*), in which females defend their own sub-territories within a male's territory

(Beletsky 1982a). In other species it may reduce the incidence of polygyny, as females behave aggressively towards other females that may look to mate with their partner (Langmore 1998); coordinate breeding activities (Ritchison 1983); or maintain pair bonds (Hovekamp 1996). Mate attraction has rarely been reported as the function of female song but has been observed in the polygynandrous Alpine accentor (*Prunella collaris*; Langmore et al. 1996). However, female song can be easily overlooked in species where individuals only sing for a small window of the breeding season or in monomorphic species where it is difficult to distinguish between the sexes (Hahn et al. 2013; Langmore 1998). Furthermore, relatively few studies have directly compared the acoustic structure of male and female song, or the behavioural and seasonal contexts in which the two sexes sing (Brunton & Li 2006; Illes 2015; Kroodsma et al. 1987; Price et al. 2008). Such comparisons may offer important insights into the function and evolution of female song in non-duetting species.

Here I investigate the structure and function of song in male and female white-throated dippers (*Cinclus cinclus,* hereafter 'dippers'). The dipper is a sexually monomorphic, riverine songbird that defends linear territories along water bodies such as rivers and streams (Tyler & Ormerod 1994) and is one of relatively few passerines of the Northern temperate zone in which both sexes sing complex songs. Dipper song has been described as 'a very sweet rippling warble' with female song 'a less sweet series of whistles and disconnected units', but no formal analysis has been carried out to date (Cramp & Simmons 1988). Crowther (2016) recently reported that many individuals are territorial and highly vocal in the winter, often in pairs, and most birds defend territories from late winter through to the end of the breeding season. In this study, I recorded and analysed the songs of individually marked birds in a wild population of dippers to compare: (1) the acoustic structure of male and female song; (2) the acoustic structure of song given in different behavioural contexts; (3) the behavioural context in which female song is given; and (4) the seasonal patterns in song production for males and females.

## 3.3 Methods

### *3.3.1 Song recording and spectrogram production*

Song was recorded opportunistically from January to July, 2014-2016, for more details of the study site, general methods, song recording procedures and sound analysis, see Chapter 2. The site was visited daily and sampling effort was spread evenly across the field site, with each territory visited at least once per week. Once an individual was located, it was given 30 minutes in which to sing; if it did not produce song in that time the observer moved onto the next individual. If a focal individual began to sing this was recorded until the bird: (1) disappeared and could not be relocated; (2) changed behaviour, e.g. to foraging; (3) remained silent for 30 minutes; or (4) had been recorded for more than an hour and a large amount of song had been collected. Complete songs were defined as having a minimum of a 0.5 s pause between them. The mean (± SD) pause between syllables was 0.23 s ± 0.05 s (range = 0.33-0.11; n = 45 individuals). The mean pause between songs was 6.50 s ± 3.70 s (range = 64.0-0.6; n = 26 individuals).

All measures of acoustic structure were obtained using 10 songs from each of 34 males and 11 females. If more than 10 songs had been recorded for a particular individual, 10 were selected after those with a low signal to noise ratio had been excluded. All 10 songs were taken from the same time period and later categorised according to behavioural context (see below). A number of complexity, frequency and temporal characteristics were then measured for each individual and compared between males and females, between males based on the context their song was recorded in, and between males and females recorded within the same context. These measures were as follows: repertoire size and versatility (complexity); maximum, minimum and

average peak frequency (frequency); and average song length, average syllables per second and song rate (temporal).

### *3.3.2 Acoustic structure*

'Versatility' is another measure of song complexity and is calculated by dividing the number of unique syllables found within one song by the total number of syllables found in that song (Järvi 1983). This measure was calculated for and then averaged across each of an individual's 10 songs. A range of frequency and temporal characteristics were also measured using Luscinia version 2.02.10.15 (Lachlan 2007). 'Maximum', 'minimum' and 'average peak frequency' were measured for each individual; maxima and minima were measured across the 10 songs, whereas average peak frequency was calculated from 50 measurements taken across each syllable in each song. 'Average song length' was calculated across 10 songs to the nearest 0.05 s and 'average syllables per second' was calculated using the number of syllables found in each song and dividing by song length, then averaging over the 10 songs analysed for each individual. 'Song rate' was calculated using the number of complete songs produced within a 30-minute period of observation for each individual during which the bird sang at least once, starting at the time when the bird was first observed. 'Total syllables' was recorded as the total number of syllables found in all 10 songs analysed for each individual.

### *3.3.3 Behavioural Context*

For every song recorded, the behavioural context was classified according to the paired status and reproductive stage of the focal individual. The three categories were defined as: (1) 'solo' songs were those of individuals recorded early on in the field season (January and early February) that had yet to be seen with a partner; (2) 'pre-breeding' songs were given by individuals that had been seen with the same partner on at least two occasions foraging, prospecting or nest-building together; and (3) 'breeding' songs were those of individuals which had paired up and had nests at the laying, incubation or nestling stage. The identity of any conspecifics other than the breeding partner of the singer was recorded whenever possible. To

avoid pseudoreplication, all songs analysed for each individual were taken from the first context in which the bird was recorded singing. The same acoustic characteristics as those compared between the sexes were compared between contexts; in this case 'repertoire size' describes an individual's use of its repertoire when singing in a particular context.

Solo females were rarely encountered more than once as they are highly mobile (Crowther 2016) and less detectable than solo males due to the high rate of song making them easier to locate. As most female song was recorded during the pre-breeding stage (see Results), the behavioural context for females was further classified as follows: (1) 'aggression' described situations in which a singing female was observed in the presence of at least one conspecific other than her partner and during which antagonistic behaviour (e.g. chasing or fighting) was observed; (2) 'nest-building' was used for songs recorded during the prospecting or nest-building phase of the breeding season; and (3) 'other' was used for the small number of songs recorded which could not be classified into either of the other categories. For each month of the season, the context in which each female was first recorded singing was recorded; sample sizes were too small for further analyses.

### *3.3.4 Seasonal trends*

To investigate the seasonal variation in song production, each individual observed as well as whether the bird sang during the observation session was noted for every day of the field season in 2014 (mean number of individuals observed per day = 4.59 ± 2.44). This year was used as it was the only year where sampling effort was consistent throughout the breeding season (Julian date, 1 = 1st January 2014).

### *3.3.5 Statistical analyses*

To compare the acoustic structure of male and female song, t-tests and Mann-Whitney U-tests were carried out for normally and non-normally distributed song characteristics, respectively. To compare the characteristics of males singing in different behavioural contexts, one-way ANOVAs were used for parametric data and Kruskal-Wallis tests for non-parametric

data followed by post-hoc Tukey tests or Dunn tests, respectively, if the initial test indicated that statistically significant differences in the results were present. To compare the characteristics of pre-breeding male song to that of pre-breeding females, t-tests and Mann-Whitney U-tests were carried out for normally and non-normally distributed data, respectively. Females classed as solo or breeding were rarely recorded singing so the sample size was not big enough for analysis across females of all song contexts. All analyses were carried out using R, version 3.2.2 (R Core Team, 2015).

## 3.4 Results

### *3.4.1 Acoustic structure and sex*

#### *3.4.1.1 Complexity characteristics*

Up to 146 different syllable types were found within one individual's repertoire, but the mean number of unique syllables found across all individuals (n = 45) was 71.53. There was no significant difference between the repertoire size of male and female birds, but female songs showed a significantly higher level of versatility than those of males (Table 1, Figure 1a), meaning that whilst males used similar numbers of unique syllables to female songs across all 10 songs, they had fewer unique syllables within individual songs.

#### *3.4.1.2 Frequency characteristics*

There was no significant difference between the minimum and average peak frequency of male and female songs (Table 1). Male song had a higher maximum peak frequency, but this difference was marginally non-significant (Table 1).

**Table 1.** A comparison of the means (± SD) of a range of complexity, frequency and temporal characteristics of song in male (n=34) and female (n=11) dippers. Significant differences ($p < 0.05$) are shown in bold; test statistics are given for t-tests (t) or Mann-Whitney U-tests (W).

| Measure | Males | Females | Test statistic (t or W) | p-value |
|---|---|---|---|---|
| Repertoire size | 68.4 ± 21.9 | 81.1 ± 29.2 | W = 145 | 0.27 |
| Versatility | 0.51 ± 0.074 | 0.57 ± 0.07 | t = -2.53 | **0.02** |
| Maximum peak frequency (kHz) | 9.84 ± 0.41 | 8.18 ± 0.24 | W = 123.5 | 0.09 |
| Minimum peak frequency (kHz) | 1.03 ± 0.40 | 1.09 ± 0.38 | W = 166.5 | 0.74 |
| Average peak frequency (kHz) | 4.85 ± 0.30 | 4.97 ± 0.19 | t = 1.46 | 0.15 |
| Average song length (s) | 6.87 ± 2.42 | 8.57 ± 4.01 | W = 145 | 0.27 |
| Average syllables per second | 3.91 ± 0.79 | 3.42 ± 0.40 | t = 2.64 | **0.01** |
| Song rate | 12.9 ± 8.70 | 19.1 ± 12.7 | W = 131 | 0.14 |
| Total syllables | 253.4 ± 88.9 | 277.0 ± 114.1 | W = 163.5 | 0.54 |

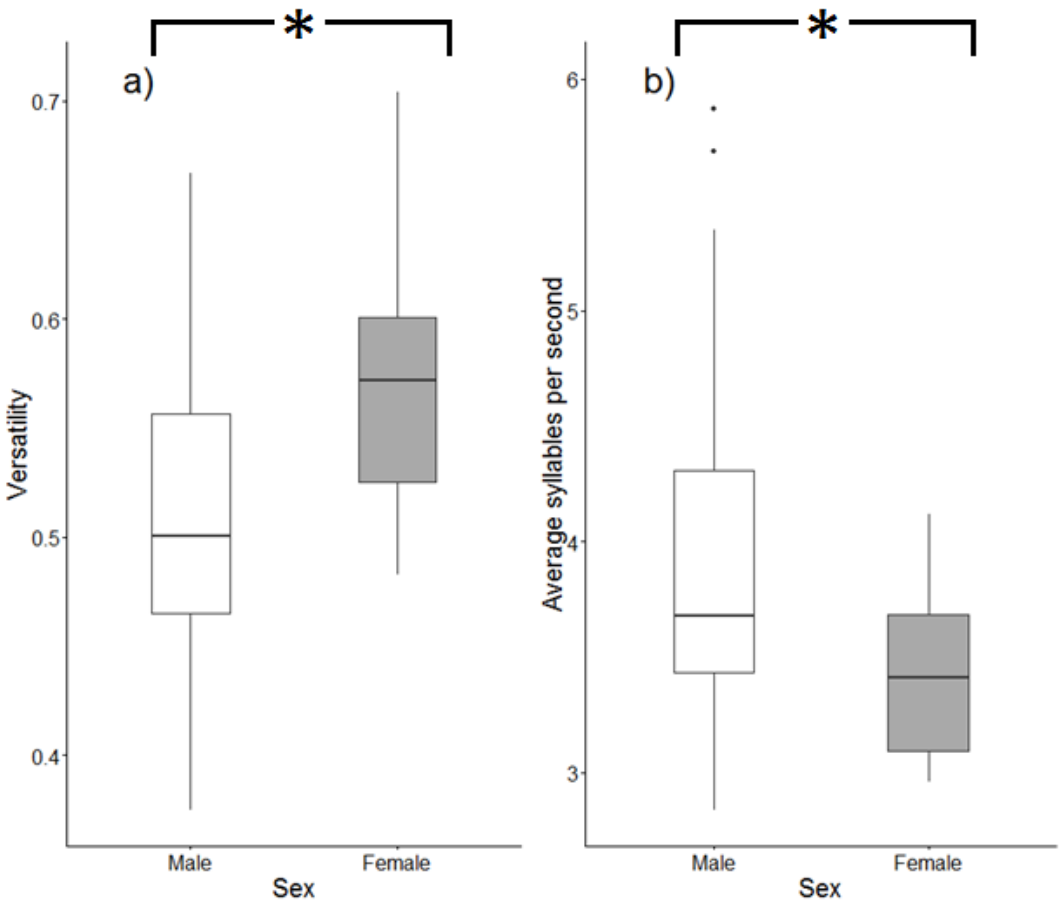


**Figure 1.** A comparison of male (white) and female (grey) song characteristics: a) versatility and b) average syllables per second. Boxes show the median, first and third quartiles; the upper and lower whiskers extend to the highest and lowest values that are within 1.5 times the inter-quartile range. Data beyond the end of the whiskers are outliers and plotted as points. Asterisks signify a significant difference between the variables.

*3.4.1.3 Temporal characteristics*

There was no significant difference in average song length, song rate or total syllables sung over 10 songs between males and females, although males sang songs with a significantly higher number of syllables per second than females (Table 1, Figure 1b).

***3.4.2 Acoustic structure and behavioural context***

*3.4.2.1 Complexity characteristics*

Males singing in different behavioural contexts used repertoires of significantly different sizes. Post hoc tests showed solo males used significantly more unique syllables in their songs than pre-breeding males ($p = 0.04$) or breeding males ($p = 0.04$; Table 2, Figure 2a). By contrast, versatility was significantly lower in breeding males than in the other groups (solo males, $p = 0.001$; pre-breeding males, $p = 0.010$, Figure 2b). Very few solo females (n=2) or breeding females (n = 1) were recorded singing, and these individuals were excluded from statistical comparisons (Table 2). There were no significant differences between the songs from pre-breeding males and females for any complexity characteristics (Table 2).

*3.4.2.2 Frequency characteristics*

There was no difference in any of the frequency characteristics between songs produced in different male behavioural contexts or between the songs of pre-breeding males and females (Table 2).

*3.4.2.3 Temporal characteristics*

There was no difference in average song length or total syllables used between songs produced in different behavioural contexts (Table 2). The number of syllables per second differed between groups, with songs of breeding males containing a higher average. Post hoc tests showed the difference between breeding male song and solo male song was significant ($p = 0.03$, Table 2, Figure 2c). Song rate was also found to differ significantly between individuals in different behavioural contexts, with the song of solo males having a significantly higher song

**Table 2**. A comparison of the means (± SD) of a range of complexity and temporal characteristics of songs produced by male and female dippers in different behavioural contexts. Statistical analyses compared song context across all male songs, and sex for the pre-breeding context. Significant differences ($p < 0.05$) are shown in bold. Sample sizes for each behavioural context are: solo male = 10, pre-breeding male = 14, breeding male = 10, solo female = 2, pre-breeding female = 8 and breeding female = 1. Significant differences ($p < 0.05$) are shown in bold; test statistics are given for t-tests (t) or Mann-Whitney U-tests (W).

| **Sex** | | **Male** | | **Analysis of male context on song** | | | **Female** | | **Analysis of pre-breeding song male vs. female** | |
|---|---|---|---|---|---|---|---|---|---|---|
| Song context | Solo | Pre-breeding | Breeding | Test Statistic | p-value | Solo | Pre-breeding | Breeding | Test Statistic | p-value |
| Repertoire size | 85.4 ± 25.1 | 61.5 ± 15.7 | 61.2 ± 17.8 | $\chi^2 = 7.96$ | **0.02** | 57.0 ± 2.83 | 82.5 ± 29.3 | 118.0 | W = 34 | 0.14 |
| Versatility | 0.55 ± 0.053 | 0.52 ± 0.07 | 0.45 ± 0.05 | F = 8.38 | **0.001** | 0.59 ± 0.05 | 0.55 ± 0.054 | 0.70 | t = -0.90 | 0.38 |
| Maximum peak frequency (kHz) | 8.42 ± 0.68 | 8.08 ± 0.52 | 8.01 ± 0.49 | $\chi^2 = 2.13$ | 0.35 | 7.77 ± 0.15 | 7.85 ± 0.28 | 7.82 | W = 47.5 | 0.59 |
| Minimum peak frequency (kHz) | 1.52 ± 0.50 | 1.59 ± 0.36 | 1.40 ± 0.42 | F = 0.60 | 0.55 | 1.53 ± 0.46 | 1.54 ± 0.46 | 1.81 | t = -0.26 | 0.80 |
| Average peak frequency (kHz) | 4.89 ± 0.42 | 4.91 ± 0.19 | 4.75 ± 0.30 | F = 0.85 | 0.44 | 4.98 ± 0.004 | 4.93 ± 0.19 | 5.30 | - | - |
| Average song length (s) | 8.33 ± 3.00 | 6.39 ± 1.79 | 6.08 ± 2.10 | $\chi^2 = 4.44$ | 0.11 | 7.43 ± | 8.02 ± 3.87 | 15.3 | W = 47 | 0.57 |
| Average syllables/s | 3.51 ± 0.37 | 3.77 ± 0.69 | 4.50 ± 0.93 | $\chi^2 = 6.84$ | **0.03** | 3.16 ± 0.10 | 3.53 ± 0.43 | 3.10 | W = 64.5 | 0.59 |
| Song rate | 19.4 ± 98.3 | 9.79 ± 7.02 | 10.7 ± 7.57 | $\chi^2 = 7.32$ | **0.03** | 13.5 ± 2.12 | 16.8 ± 9.32 | 49.0 | W = 30.5 | 0.09 |
| Total syllables | 283.6 ± 90.9 | 227.6 ± 64.2 | 259.2 ± 112.8 | $\chi^2 = 2.74$ | 0.25 | 228.0 ± 60.8 | 270.3 ± 118.4 | 429.00 | W = 46 | 0.52 |

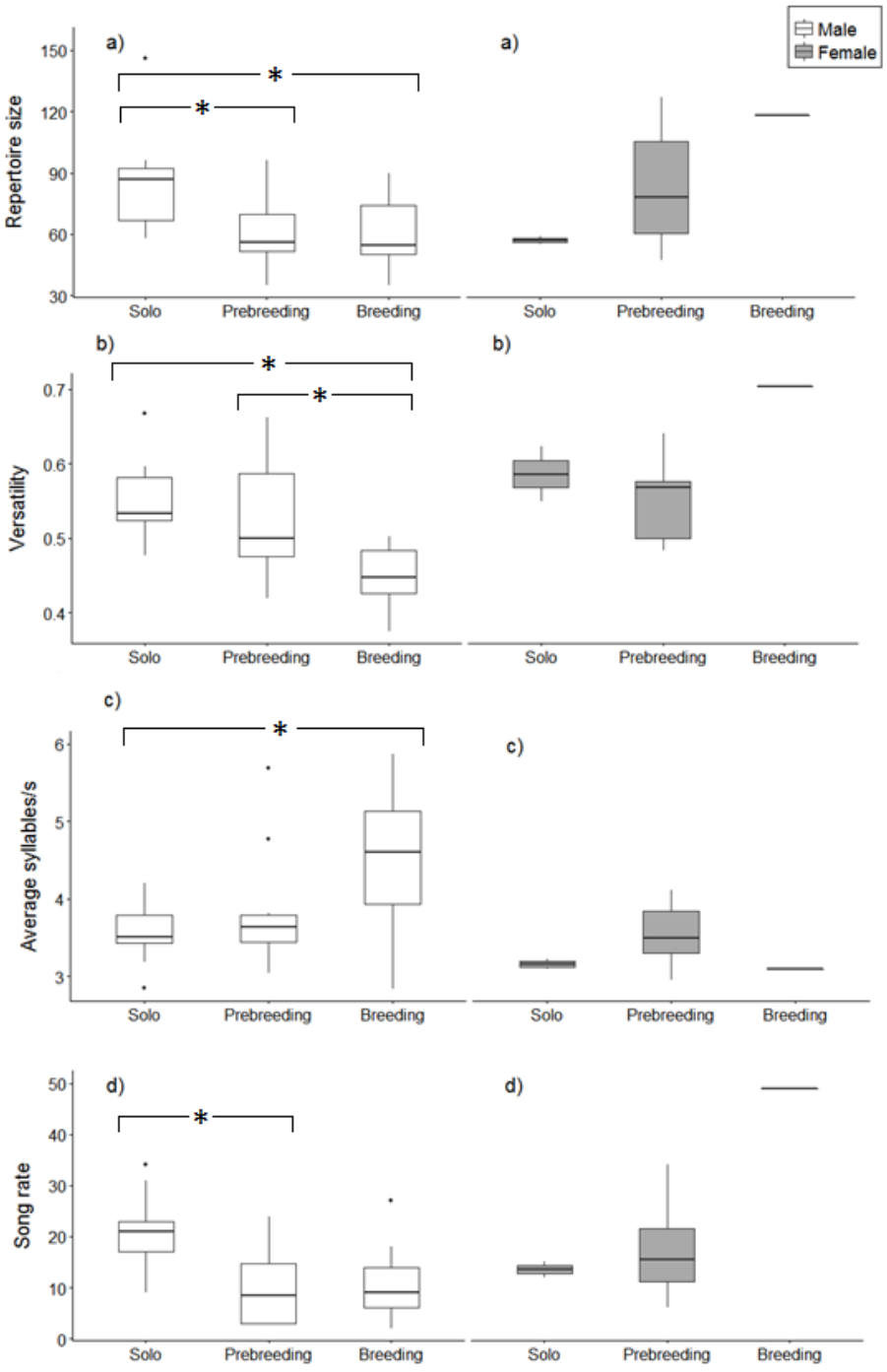


**Figure 2.** A comparison of male (white) and female (grey) song characteristics between different behavioural contexts for: a) repertoire size, b) versatility, c) average syllables per second and d) song rate. The box shows the median, first and third quartiles; upper and lower whiskers extend to the highest and lowest values that are within 1.5 * inter-quartile range. Data beyond the end of the whiskers are outliers and plotted as individual points. Asterisks signify significant differences between contexts.

rate than that of pre-breeding males ($p = 0.03$, Table 2, Figure 2d). There were no significant differences between the songs of pre-breeding males and females in any of the temporal characteristics (Table 2).

### *3.4.3 Female song context and seasonal trends*

Female song was frequently observed during aggressive encounters in the early part of the season (Figure 3). In instances where all individuals were identified, 8/10 of the observations of aggressive female song involved an intruder female present either on her own (5/10) or with her partner (3/10). Some females also sang during the nest-building period and occasionally in other contexts, but song was less frequently recorded later in the season (Figure 3). The proportion of observed males which were recorded singing peaked in the early part of the season but then remained at a relatively high level at all other times across the season (Figure 4a). The seasonal decline in singing by females was also apparent from the decrease in the proportion of observed females which were recorded singing as the season progressed (Figure 4b).

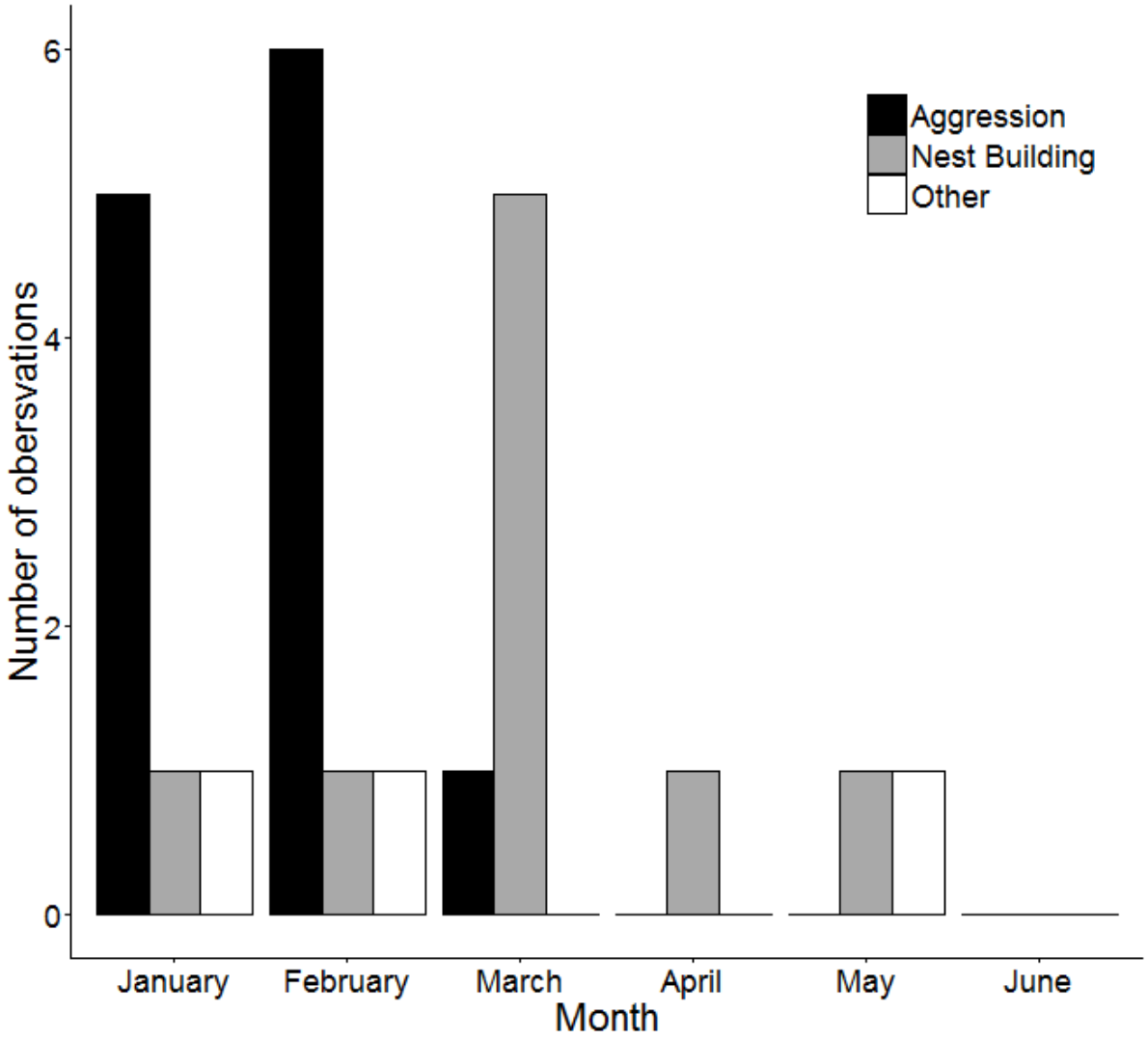


**Figure 3.** The number of observations of females singing in different behavioural contexts during each month of the breeding season: "aggression" (black) describes females within their own territory which sang in the presence of an intruder i.e. not their partner; "nest-building" (grey) describes females recorded singing whilst prospecting or building a nest with their partner; and "other" (white) describes females which sang in all other contexts.

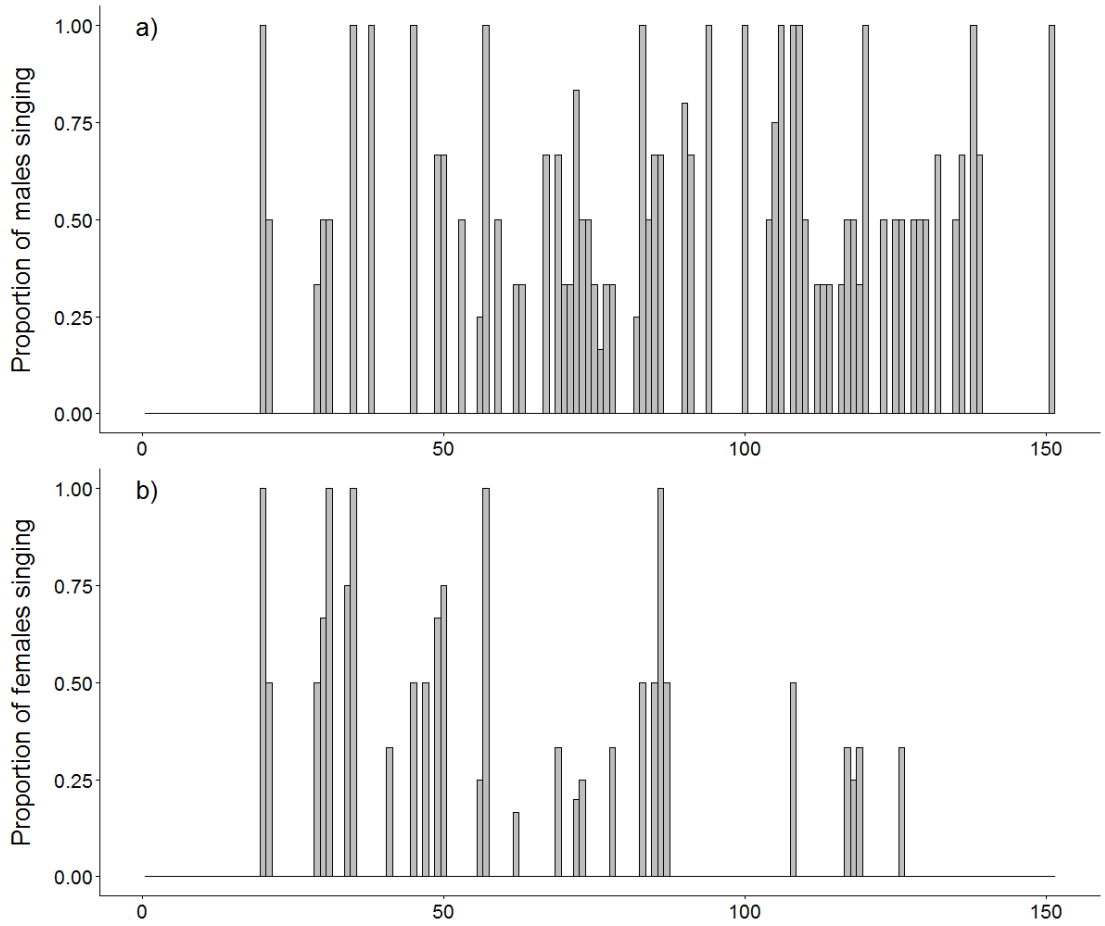


**Figure 4.** The proportion of observed individuals recorded as singing daily across the breeding season for a) males and b) females.

## 3.5 Discussion

Male and female song was similar in structure and frequency range; however, the difference in maximum peak frequency was approaching significance with males singing higher frequencies and having a larger range of frequencies within their vocal repertoire. This could explain why females are reported to have a more scratchy, less melodious quality to their song (Cramp & Simmons 1988). Female song had significantly higher levels of versatility, whereas male song had a higher number of syllables sung per second. It is possible that these subtle differences in song characteristics code different information about the sex of the singer and, in support of this, studies have found that in species where songs and calls of both sexes sound very similar to the human ear, there is usually an acoustic dimorphism which is thought to facilitate sex discrimination (Curé et al. 2011; Gahr & Guttingery 1986; Nuechterlein & Buitron 1992; Yamaguchi 1998).

Solo males were found to have higher song rates and larger repertoire sizes, whereas breeding males use a larger number of syllables per second, but with less versatility to their song. These differences in characteristics according to song context have been reported in other species; for instance, zebra finches (*Taeniopygia guttata*) produce directed and undirected song which have differences in length, tempo, and variability, and it has been shown that females are more strongly attracted to directed song (Woolley & Doupe 2008). In blue grosbeaks (*Guiraca caerulea*) it was found that during periods of female fertility, males increased the proportion of distinct song variants, had higher versatility and elevated levels of syntax consistency within their songs as well as singing for longer during the dawn chorus at an increased rate (Ballentine et al. 2003). Motivational state of the individual could be the explanation behind the variability in songs within dippers, with the difference in song effort and complexity between solo male and breeding male song suggesting that males use song early on in the season for mate attraction. Singing is an expensive behaviour, not just due to the energetic costs of performance

related traits, but indirect costs such as taking time out of the feeding budget in order to sing (Gil & Gahr 2002). There is also an increased risk of predation as some predators have been shown to locate their prey by eavesdropping on their mating calls (Mougeot & Bretagnolle 2000). Presumably, the cost of singing at a higher rate in solo males is balanced by the benefits of investment in future reproductive fitness by finding a mate.

Most incidences of female song occurred in the presence of another female or intruding pair; production of androgens could have an influence on the prevalence of female song by increasing the levels of intra-sexual aggression (Arcese et al. 1998). This has also been seen in yellow warblers (Hobson & Sealy 1990), in which it is thought female song may decrease the incidence of intraspecific brood-parasitism or polygyny, with females using aggressive song to keep competition away. The female song context 'other' demonstrates that song also occurred in non-aggressive instances. In both recorded cases, song occurred in the presence of a male at times when egg laying may have been imminent. One female was observed singing in May whilst accompanied by her partner, with two 18 day old chicks in the nest. It is possible this female may have been advertising her fertility to her mate in preparation for a second clutch (Baptista et al. 1993). The other female sang whilst within her territory range in the company of a male that was not her partner, singing and displaying to him, and may have been advertising her fertility to secure an extra-pair copulation (Baptista et al. 1993). Female song used to advertise fertility for mate attraction has been seen in the polygynandrous Alpine accentor (Langmore et al. 1996), but has otherwise rarely been recorded.

Male dippers sing throughout the breeding season, but female song peaks during late January to early February and shows a steady decline until the end of March when egg laying commences. It has been hypothesised that the seasonal pattern with which singing occurs often reflects the role of song (Brunton & Li 2006). Here, the results indicate that female song was almost exclusive to two periods within the breeding season. First, from January to early February whilst territories were being established between neighbours, when song may be used to defend a territory or a mate from an intruder. In monogamous species with year-round

territoriality there is high selection pressure for joint territorial defence, and therefore female song (Farabaugh 1982). Crowther (2016) reported that territorial dippers remained in heterosexual pairs over the winter, and female song may therefore play a role in territory defence. Only one instance of solo female song was recorded in early January; no other individuals were nearby and this female had not been seen with her mate yet that year. In this instance, the main function of the song is likely to have been due to territorial defence as this female had been caught in the same location earlier in the winter and also went on to breed within this territory; however, mate attraction cannot be ruled out. In mate removal experiments with bay wrens (*Thryothorus nigricapillus*), a duetting species, females were able to defend their territories with song once males had been removed (Levin 1996). In other species, each sex may only defend against same-sex intruders, therefore territory defence as a pair is better than individual defence over the winter (Langmore 1998). This behaviour has been observed in northern cardinals (*Cardinalis cardinalis*), where female song did not deter the intrusion of a new male but was thought to deter other females (McElroy & Ritchison 1996).

The second major period in which female dippers were recorded singing was from late February to the end of March during prospecting and nest-building. Song at this time may play a role in pair bonding or breeding coordination. Other species in which females sing during specific breeding activities include: black-headed grosbeaks (*Pheucticus melanocephalus*), which sing during nest-building and incubation (Ritchison 1983); song sparrows (*Melospiza melodia*) in which song almost exclusively occurs during the period immediately prior to nest-building (Arcese et al. 1998); yellow warblers (*Dendroica petechia*) in which song occurs as pairs settle and then declines once laying begins (Hobson & Sealy 1990); and white-crowned sparrows (*Zonotrichia leucophrys*), the females of which sing during the period of maximum oestrogen production prior to nest-building and egg laying, and also during times of conflict when androgens are produced because of aggressive interactions with other females (Baptista et al. 1993).

Female song may be used in dippers to advertise ownership over a territory or mate to a rival female, provide the male with information on her reproductive state or even to attract a mate or extra-pair fertilisation. It is likely that the frequency of song production declines due to energetic constraints as parental care is required, particularly in brooding females (Brunton et al. 2008). Singing on or near the nest may also compromise offspring survival by making nests more conspicuous to predators (Kleindorfer et al. 2016).

## 3.6 Conclusion

This investigation of the acoustic characteristics, behavioural context and seasonality of male and female song in dippers is a useful platform for testing potential hypotheses of song function in this and other monogamous species. Whilst the song of sexes is structurally similar, they vary with context. Males sing throughout the breeding season, and this coincides with activities such as pair bonding, breeding coordination and territory defence. However, song in males can be more complex early in the season when they are unpaired and the primary function is likely to be mate attraction. In females, song is almost entirely confined to the early part of the breeding season when territories are being established and nest-building begins. It therefore appears to be primarily used for mate or territory defence, but may also play a role in pair bonding and fertility advertisement. Playback experiments are now required to explore these ideas further. Furthermore, it is still unknown whether song is a sexually selected trait in females (Pavlova et al. 2005) and analyses of the relationship between song complexity and fitness would shed further light on the function and evolution of the female song.

# Chapter 4

# Playback experiment investigating song function and territoriality in pairs

## 4.1 Abstract

Territorial defence is one of the main functions of birdsong, particularly in those monogamous species in which both sexes sing to coordinate the joint defence of resources. Whilst female song is most prevalent in tropical and Australasian species, there are examples from birds living in the temperate zone but the function of female song in these species has received comparatively little attention, and the relative role of males and females in defending their territory remains unclear. To address this, playback trials were conducted on pairs of dippers within a marked, long-term study population. Focal males and females were analysed for their responses to playback song according to (i) the sex of the simulated intruder and (ii) the timing of the playback trial relative to the onset of breeding (i.e. before or after egg laying). The sex of the simulated intruder and the timing of the trial did not affect either sex's likelihood of responding, measured as singing in response to the playback or approaching towards the speaker. This suggests that unfamiliar female song is perceived in a similar way to unfamiliar male song, irrespective of whether or not breeding has commenced, thereby providing evidence for a territorial function of female song. A higher proportion of focal males than females responded to the playback of male song, and males were also significantly quicker to respond to the simulation of an intruder than females. Furthermore, in trials where both pair members

approached the speaker in response to the playback, the male was always first to respond. These results suggest that males may take the dominant role in defence within the pair, which is likely due to females investing more time and energy in egg production, incubation and brooding.

## 4.2 Introduction

The two main functions of birdsong are mate attraction and territorial defence (Catchpole & Slater 1995; Catchpole 1996). Individuals that sing at a higher rate, using larger repertoires or with higher levels of complexity, can pair up earlier (Catchpole 1980); attain better quality territories (Buchanan & Catchpole 1997; Manica et al. 2014); have longer territory tenure (Hiebert et al. 1989; Potvin et al. 2013); and thus achieve greater reproductive success (Lambrechts & Dhondt 1986; McGregor et al. 1981; Potvin et al. 2013). Earlier pairing gives them the opportunity to start breeding earlier, which can mean better resources for rearing offspring or improved chances of having a second brood (Seward et al. 2014; Verboven & Verhulst 1996). However, while song function has been studied extensively in males, most research on female song has focussed on the species in which it is most prevalent: those that duet (Hall & Peters 2008; Mennill & Rogers 2006; Rogers et al. 2006; Topp & Mennill 2008) and those that live in the tropics and Australasia (Brunton & Li 2006; Odom et al. 2014).

Duetting, where both members of a mated pair sing in combination with each other, either synchronously or alternately, is commonly found within monogamous species inhabiting the tropics (Hall & Peters 2008; Langmore 1998; Thorpe et al. 1972). There, year-round territoriality and permanent pair-bonds mean the roles of the sexes converge resulting in female song (Morton 1996). Within duetting species, the functions of female song are often similar to

those of male song, sometimes occurring in a cooperative form with female song matching male song for joint defence of a territory or parental care (Hall & Peters 2008). In other cases the song differs between males and females, with each sex singing for territorial or mate defence against same-sexed individuals (Morton 1996). However, far less is known about the function of female song in non-duetting species (Slater & Mann 2004).

Different mating systems appear to coincide with different functions of female song. Females in polygynous species can defend either a physical space (Beletsky 1982a) or the parental investment they may receive from their partner (Yasukawa & Searcy 1982), and in these instances, song has been shown to deter other females, thus reducing the incidence of extra-pair copulation or polygyny (Langmore & Davies 1997). In monogamous species with year-round territoriality, it is thought that there is high selection pressure for joint territorial defence (Farabaugh 1982; Hoelzel 1986; Langmore 1998). Joint defence is more efficient than solo defence and each individual of the pair contributes to increase their own fitness (Langmore 1998; Morton 1996). In some cases, a female's defence of her partner can result in exclusive mating access or a reduction in polygyny (Hoelzel 1986). By contrast, mate defence by males not only increases their chance of paternity (Topp & Mennill 2008) but can also increase their likelihood of extra-pair mating (Hoelzel 1986).

Joint territorial defence has been the subject of many playback trials, with intrasexual aggression being prevalent among the responses. In studies of bell birds (*Anthornis melanura*; Brunton *et al*. 2008), rufous-and-white wrens (*Thryophilus rufalbus*; Topp & Mennill 2008) and barred antshrikes (*Thamnophilus doliatus*; Koloff & Mennill 2011), males preferentially responded to the playback of male song over female song; and in rufous-and-white wrens (Topp & Mennill 2008), superb fairy-wrens (*Malurus cyaneus*; Cain & Langmore 2015) and house wrens (*Troglodytes aedon*; Krieg & Getty 2016), females responded more to the playback of female song than male song. However, few playback experiments which have investigated the role each sex plays in territoriality have tested all possible responses. In some cases, for example, only one sex of intruder was simulated (Brunton et al. 2008; Cain & Langmore 2015),

intruders of both sexes were simulated but only the responses from one sex were observed (Krieg & Getty 2016), the stimuli used were a duetting pair (Topp & Mennill 2008) or the playback trial was only conducted during one part of the breeding season (Koloff & Mennill 2011). Responses may differ before and after breeding if female song is used only for mate defence as females may be less inclined to defend a partner once they have offspring together, whereas if the function of female song is for territorial defence then the response to an intruder would not be expected to change across the breeding season (Cain & Langmore 2015). More experiments involving all combinations of responder and intruder by sex are therefore needed to further understand the function of female song and the relative contributions of males and females to territorial defence.

The white-throated dipper (*Cinclus cinclus*, hereafter 'dipper') is a sexually monomorphic, riverine songbird in which both sexes defend linear territories along rivers and streams and sing throughout much of the year (Tyler & Ormerod 1994; Chapter 3). In Chapter 3, I established that singing by females was observed much less frequently once eggs had been laid, presumably due to the impact that parental care has on their time and energy budget (Brunton & Li 2006) and the effect that singing could have on the risk of offspring predation (Kleindorfer et al. 2016). This decline in song frequency suggests that female song is unlikely to be used for coordinating parental care of offspring, but rather for territorial defence or coordination of breeding activities. A role in mate attraction also seems unlikely because song is most frequent in females which have already found a mate (Chapter 3). Here, I build on this work by investigating the responses of both sexes to playback of unfamiliar male and female song (i.e. simulated intruders) in order to test whether: (1) male and female responses to playback of unfamiliar intruders differ with the sex of the intruder; (2) male and female responses to playback of unfamiliar intruders differ before and after breeding has commenced; (3) members of a pair respond differently to the simulation of an intruder (regardless of breeding stage); and (4) the latency to respond differs between members of a pair when both sexes respond.

## 4.3 Methods

### *4.3.1 Study site and species*

This study was conducted on an individually marked population of 40-50 pairs of dipper in the River Lune catchment near Sedbergh, Cumbria, UK (54°323'N, 2°528'W); the population is the subject of a long-term study and a pedigree has been established from observational and ringing data (see Chapter 2). The data presented in this chapter were collected from February to June 2016. In this population, the first pairs begin nest-building in February; a variable number have second broods with the last chicks fledging in early July.

### *4.3.2 Song recordings*

Songs used for playback were recorded opportunistically between January 2014 and June 2015. For sound recording and analysis methods, see Chapters 2 & 3.

### *4.3.3 Playback file construction*

Eight songs from each of five males and five females were used to create playback files with which to simulate an unfamiliar intruder to any pair's territory within the study site. Care was taken to select recordings based on clarity to maximise signal-to-noise ratio during playback. All songs were also high pass filtered, using Avisoft, at 1 kHz to remove low frequency background noise (e.g. the sound of the river); this threshold was chosen because dipper repertoires have been found to contain elements as low as 1.03 kHz (Chapter 3). One playback file per individual was created each consisting of a looped sequence of the eight songs. Pauses between songs were determined using the average duration between songs for that individual, measured across a sample of 10 songs.

### *4.3.4 Playback design*

All trials took place between dawn and noon on days when there was little or no wind to interfere with the sound. Breeding pairs were considered to be 'established' when they had been observed foraging or nest-building together on two or more separate occasions; once established, the trials could commence. Each of 11 focal pairs underwent four trials, two before egg laying ('pre-incubation') and two after the pair had reached the nestling stage and were no longer brooding ('post-incubation'). Three additional pairs underwent the pre-incubation trials but did not go on to breed. Trials within the same breeding stage were separated by at least one day, one involving playback of male song and the other playback of female song. Focal pairs were assigned one male and one female playback file for each breeding phase using a random number generator. For the first trial, each playback file was given a number between one and ten. Playback files were removed from the random draw if they contained songs of an individual considered to be familiar to the focal pair; individuals were classified as familiar if they had been observed on the same river as the pair or if they were a close relative of either bird (i.e. a parent, offspring or sibling). The playback file for the second trial was selected from playback files of the opposite gender to the first, labelled 1-5 and again drawn using a random number generator. The third and fourth trials followed the same pattern, each time removing the playback files played in previous trials. This system ensured that the sex of the first playback file in each pair of trials was randomised for each focal pair, thereby removing order effects.

For the two pre-incubation trials, the speaker was situated in a part of the territory near to where the pair would typically forage together that was also visually accessible to the observer. All playbacks were conducted by the same observer for consistency in measures. For the post-incubation trials, the speaker was placed 10-20 m away from the nest. For all trials the distance between the speaker, focal birds and observer varied according to the landscape, but the birds were always between 10-30 m away from both the speaker and observer. Playback of song was broadcast through a FoxPro Inferno speaker at a volume which best mimicked natural song; this was determined in preliminary trials by comparing the amplitude of 10 playback

songs to that of 10 randomly chosen songs recorded from the same distance (song: $\bar{x}$ amplitude = 34.72 ± 9.14, 0.11 – 49.99; playback: $\bar{x}$ amplitude = 38.16 ± 4.83, 23.92 – 49.89; all recorded at 10 - 15 m, measured using Luscinia). Before each trial the speaker was positioned (Figure 1.) and checked for volume by playing a control sound: the pre-programmed 'juvenile goat', which was least likely to provoke an unwanted response from the focal pair as other options were bird distress calls. The control sound was followed by a five-minute period of quiet. Playback trials commenced once birds were present near the speaker within clear view of the observer and were noted to be feeding or resting. In all trials, at least one focal bird looked at the speaker during playback. Both members of the pair were usually present, but some pairs rarely fed or rested together during the experiment and trials were therefore conducted with a single focal bird (n = 10 pre-incubation trials, n = 9 post-incubation trials); in these cases, the female's presence was prioritised where possible in order to balance sample sizes as lone males were more frequently encountered. Two minutes of continuous song playback were followed by two minutes of quiet; preliminary trials indicated that these timings were ample for the focal individuals to respond before resuming 'normal' behaviours e.g. foraging/resting.

Responses of focal birds during each four-minute trial were recorded as behavioural or latency measures in seconds from the start of the trial commencing. Behavioural responses were (1) whether the focal bird approached (i.e. hopped or flew) towards the speaker and (2) whether it sang. The latency to respond was recorded by measuring the time (in seconds) until the focal bird either first approached the speaker or first sang i.e. the latency to respond in either way. Sample sizes were too small to analyse the latency to approach the speaker and latency to sing separately. Behavioural responses of each individual were documented throughout the trial using a voice recorder to ensure no behaviours were missed and all timings of responses were accurate relative to the start of the trial.

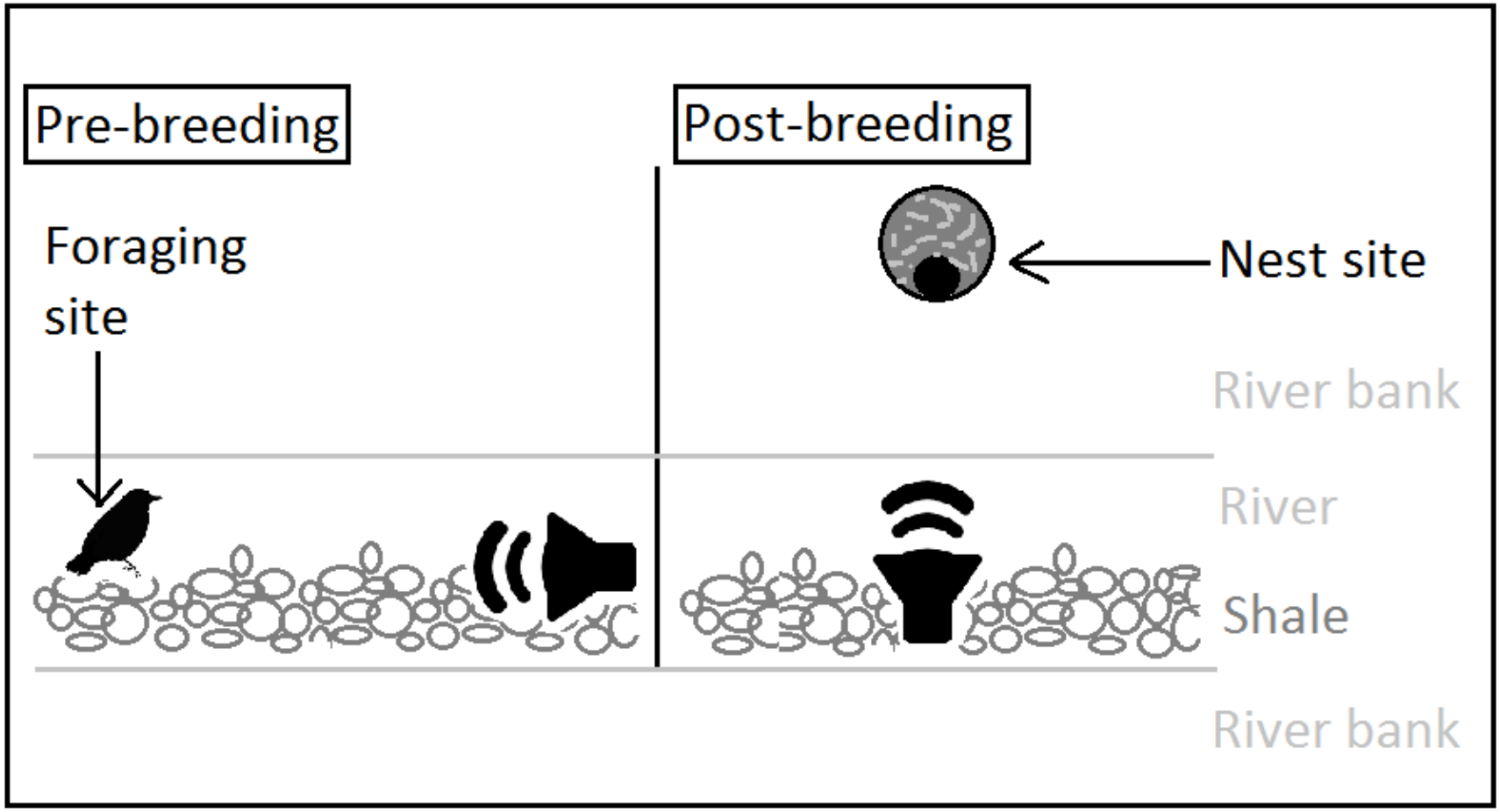


**Figure 1.** Diagram of set up for playback experiment, differing for pre- and post-breeding trials.

### *4.3.5 Statistical analyses*

All analyses were carried out using R, version 3.2.2 (R Core Team, 2015). To investigate the differences in responses of individuals to male and female song playback, the proportion of individuals responding during each breeding stage was measured: male (n = 6) and female (n = 8) pre-incubation responses to male versus female song, and male (n = 7) and female (n = 8) post-incubation responses to male versus female song. Individuals' responses were also investigated to test whether they differed between breeding stages by comparing the proportion of responses to playback pre- and post-incubation (male response to male song: n = 8; male response to female song: n = 7; female response to male song: n = 8; and female response to female song: n = 10). To investigate whether male or female partners were the more likely to respond during playback of male or female song, irrespective of breeding stage, the number of males and females responding to at least one trial was compared (playback of male song: n = 8; playback of female song: n = 7). All paired comparisons were carried out using McNemar's exact tests.

To investigate whether males and females differ in their latencies to respond, the fastest response times from each individual for playback of male song (n = 10 males, n = 3 females) and female song (n = 8 males, n = 7 females) were compared; latencies to respond to male song were not statistically analysed due to the small sample of female subjects. The quickest response by males and females to any trials were compared irrespective of stimulus (n = 14 males, n = 9 females). These data were non-normally distributed and so latencies to respond to female song and to playback overall were compared using Mann-Whitney U tests. The latency to respond was also compared between pair members in trials during which both members responded by approaching the speaker (n = 5). These data were analysed using a Wilcoxon signed rank test due to the small sample size.

## 4.4 Results

In 53% of trials (=25/47), at least one individual in the pair responded to the playback by approaching the speaker, singing or both. In 17% of trials, at least one individual in the pair approached the speaker, 9% had at least one individual sing and in 28% of trials there was both song and movement towards the speaker in response to the playback.

### *4.4.1 Responses by breeding stage*

There was no significant difference in the proportion of individuals that responded during pre-incubation playback of male or female song for either focal males (male song: 67%, female song: 50% n = 6, $p \approx 1$) or focal females (male song: 38%; female song: 37.5%; n = 8, $p \approx 1$). Similarly, during post-incubation playback of male and female song, there was no significant difference between the proportions of individuals that responded in either males (male song: 43%; female song: 29%; n = 7, $p \approx 1$) or females (male song: 13%; female song: 25%; n = 8, $p \approx 1$).

### *4.4.2 Pre- versus post-incubation responses*

There was no significant difference in the proportion of males that responded to playback between pre- and post-incubation trials either for male song (pre: 50%; post: 38%; n = 8, p = 0.69) or female song (pre: 43%; post: 43%; n = 7, $p \approx 1$). There were also no significant differences between pre- and post-incubation trials in the proportion of females that responded to playback of male song (pre: 38%; post: 13%; n = 8, p = 0.50) or female song (pre: 50%; post: 20%; n = 10, p = 0.38).

### *4.4.3 Pair responses*

There was no significant difference in the proportion of males and females which responded to playback of male song (males responded = 75%; females responded = 35%; n = 8,

p = 0.25, figure 2a) or female song (males responded = 57%; females responded = 57%; n = 7, p ≈ 1, Figure 2b).

#### *4.4.4 Latency to respond*

Males took less time to respond than females in response to playback of male song, but this difference could not be tested due to the small sample size (Table 1, Figure 3a). There was no difference in the time it took males and females to respond to the playback of female song (Table 1, Figure 3b). Overall, however, males responded significantly quicker to playback than females (Table 1, Figure 4), and in every instance where both members of the pair approached the speaker, the latency was shorter in males; this result was marginally non-significant (n = 5, V = 15, p = 0.06; Figure 5).

## 4.5 Discussion

No differences between males and females were found in the responses to simulated intruder song of either sex during pre-incubation or post-incubation trials. Whilst the sample sizes are small, these results suggest that male song and female song are equally likely to provoke a response in both males and females, irrespective of whether or not breeding has commenced. Territorial defence is conducted by both sexes (Tyler & Ormerod 1994) and it may be that all unfamiliar song (i.e. not that of a partner or neighbour) is treated as intruder song by established pairs. This would support the suggestion of a territorial function for female song; other functions would be more likely to elicit sex-specific responses or different responses before and after the start of breeding.

Post-breeding responses were generally lower, but the difference was marginally non-significant. This trend suggests that pairs may invest more in territoriality prior to the onset of

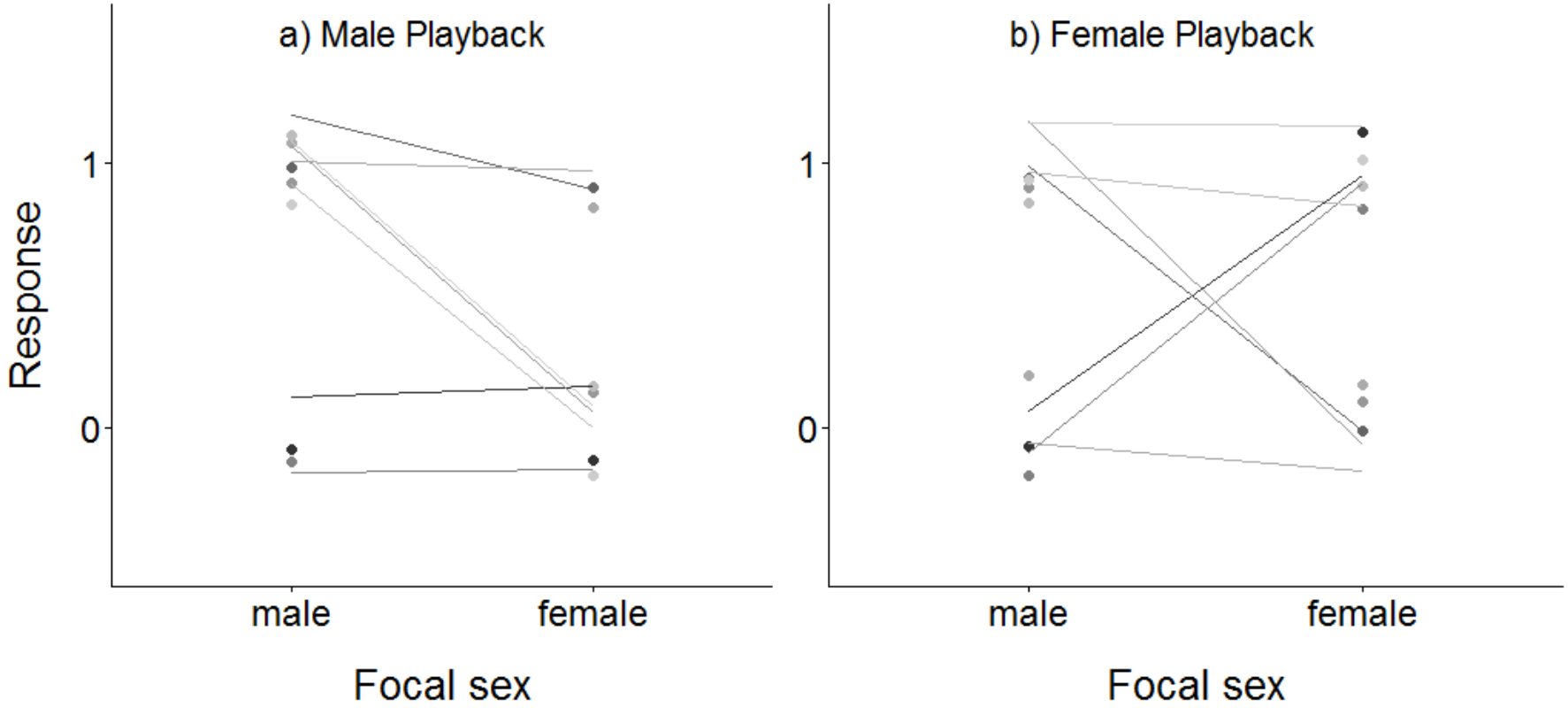


**Figure 2.** Male and female responses to the playback of a) male song and b) female song. Each point represents and individual, individuals within the same pair are colour coordinated and lines link individuals within a pair. All points and lines are jittered to view all data points.

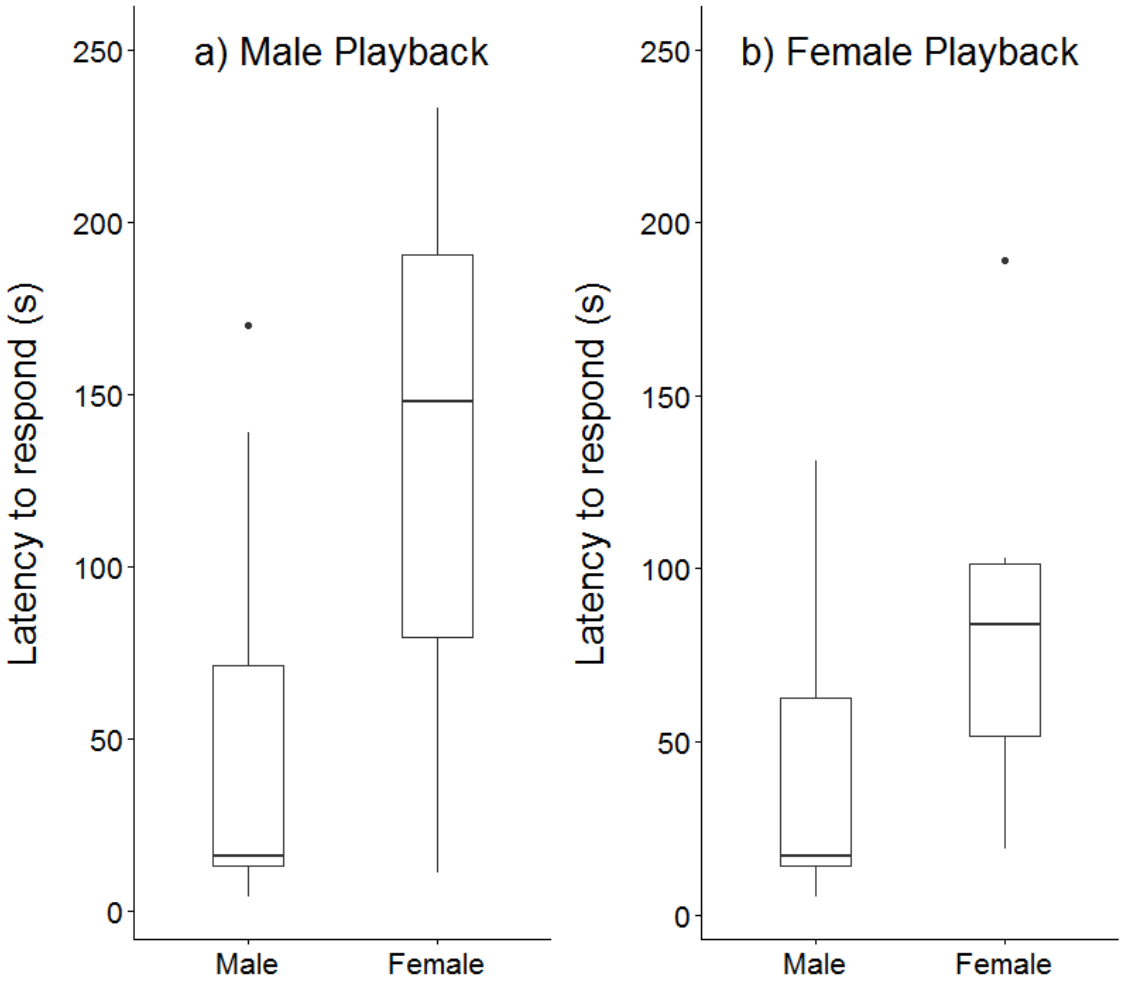


**Figure 3**. Male and female latencies to respond (approach the speaker, sing or both) during playback of a) male song (male: n = 10; female: n = 3); and b) female song (male: n = 8; female: n = 7). Boxes show the median, first and third quartiles, and the upper and lower whiskers extend to the highest and lowest values that are within 1.5 times the interquartile range. Data beyond the end of the whiskers are outliers and plotted as individual points.

**Table 1**. A comparison of the mean (± SD) response time in seconds for each individual's quickest response to a given stimulus, measured from the start of the playback for all trials that elicited a response (n = 14 males, n = 9 females). Significant differences ($p < 0.05$) are shown in bold; test statistics are given for Mann-Whitney U-tests (W).

| **Playback song sex** | **N** | | **Focal Male** | **Focal Female** | **Test statistic** | **p-value** |
|---|---|---|---|---|---|---|
| | **Male** | **Female** | | | | |
| Male | 10 | 3 | 49.3 ± 60.68 | 130.66 ± 112.01 | - | - |
| Female | 8 | 7 | 42.13 ± 45.25 | 85.43 ± 54.89 | W = 21 | 0.64 |
| Both | 14 | 9 | 42.79 ± 53.57 | 98.56 ± 77.01 | W = 95.5 | **0.04** |

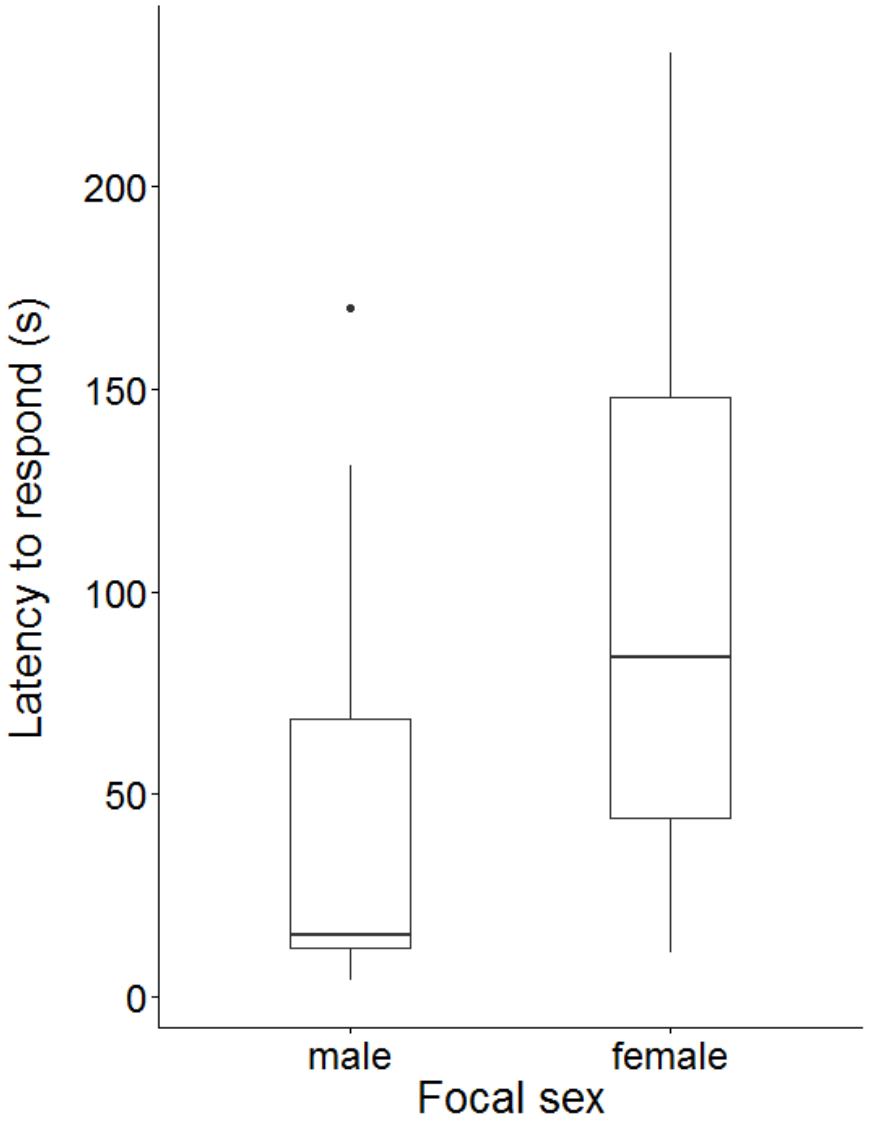


**Figure 4**. Male and female latencies to respond (approach the speaker, sing or both) to playback. Values are taken from fastest response to either male or female song. Boxes show the median, first and third quartiles, and the upper and lower whiskers extend to the highest and lowest values that are within 1.5 times the interquartile range. Data beyond the end of the whiskers are outliers and plotted as individual points.

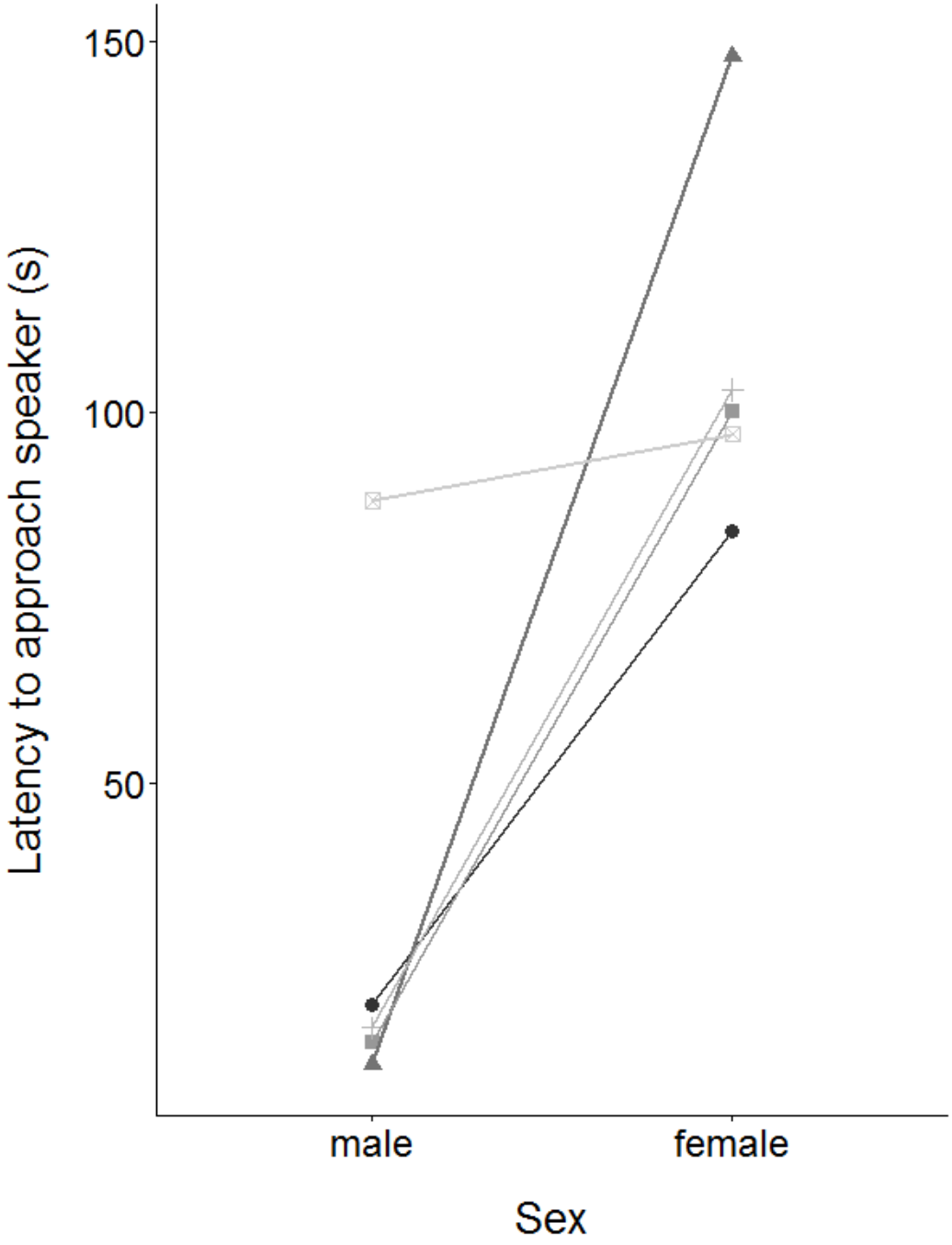


**Figure 5**. Male and female latencies to respond by approaching the speaker, measured in seconds from the start of the playback for trials during which both members of a pair responded. Each point represents an individual; pairs are linked by lines and presented using the same shape and colour.

laying, possibly because this is before a pair has made a mutual investment in rearing chicks together. Vigilance could be higher during this time for males to ensure paternity and for females to reduce the incidence of polygyny (Hall & Peters 2008; Langmore 1998; Marshall-Ball et al. 2006), but more data are needed to confirm this. In this population, the pre-incubation period is a time when both individuals actively defend their resources from any potential intruders including unpaired 'floaters' (Crowther 2016) and, at territory boundaries, their neighbours (Tyler & Ormerod 1994). These behaviours, however, become less frequent as the season progresses (Tyler & Ormerod 1994) when floaters also are less common (Crowther 2016), and it is possible that a trial conducted towards the end of the breeding season would produce even fewer responses.

Although sample sizes are small, it is worth noting that there were no instances of focal females singing in response to male song in any of the experimental trials, suggesting that females do not commonly behave aggressively towards male intruders. This, together with the observations that females responded to a higher number of female than male songs, and responses to female songs had shorter latencies, may indicate that territorial aggression in females is typically intra-sexual. Females may be responding primarily to keep other females from mating with their partner and to defend the parental care they receive from him (Yasukawa & Searcy 1982). Females may not perceive intruder males as a direct threat to their resources unless they interfere with breeding, for example by removing chicks from the nest. This has been seen on two occasions within the study population; in both cases the resident male had been missing for a few days, and the intruder male began to remove lining from nests with mature chicks in while the female remained close by, singing loudly throughout. It is therefore plausible that females, whilst having the ability to defend the territory, may reserve their responses for individuals likely to directly affect their resources.

When all responses were pooled, males reacted significantly more quickly to the simulation of an intruder than females, and in trials where both individuals had approached the speaker; males were always the first to approach, even when one male responded after over a

minute of playback. This supports the suggestion that males take a dominant role in territorial defence and this could be due to the breeding constraints on females (Brunton & Li 2006). There are very few incidences of individuals of any species singing whilst on the nest, and where vocalisations have been observed it is usually for coordination of biparental care to maximise offspring fitness (Leonard 2008). However, singing on the nest can also increase nest parasitism and predation rates and therefore remaining silent around the nest can reduce the incidence of these occurrences (Kleindorfer et al. 2016). In the case of dippers, males will occasionally produce song when arriving at the nest to feed chicks, but it is rare for the female to vocalise whilst incubating or brooding (personal observations). This corresponds with what has been observed in superb fairy-wrens, where female song rate predicted nest predation rates mainly caused by the females increased proximity to the nest (Kleindorfer et al. 2016).

## 4.6 Conclusion

Overall, the results presented here suggest that female song is perceived the same as male song by both sexes when the presence of an intruder is simulated using playback. The evidence also suggests that males were more likely to respond to an intruder and that their responses were faster, illustrating their dominance in the role of defence. However, the fact that females do sometimes respond indicates that they may also play a role in territorial defence; this may be particularly important in dippers due to their year-round territoriality in stretches of river that can be up to 1 km in length. Sample sizes were relatively small in this experiment, and further work is clearly needed to better understand the roles of males and females in territoriality and the function of female song. In particular, future trials should test other aspects of song such as individual or partner recognition and should be conducted at other times of the year.

# Chapter 5

# The effect of early life conditions on song characteristics in males

## 5.1 Abstract

Song complexity and singing frequency in male birds are shaped by female choice; they signal male quality because song is costly to develop and produce. The timing of song learning and the development of the brain structures involved occur during a period when chicks are exposed to a number of potential stressors. The quality and quantity of song produced by adults may therefore reflect the level of stress experienced during early life, a theory known as the 'developmental stress hypothesis'. This was tested using song recordings and life-history data from an individually marked, long-term study population of wild dippers. The extent to which early life conditions predict adult song characteristics was investigated using natal brood size as a measure of sibling competition, the rate of provisioning by parents as a proxy for nutritional stress, and tarsus length as a measure of body condition. There was a small but significant association between natal brood size and repertoire size, with nestlings from smaller broods producing more complex song, but no correlation between repertoire size and either tarsus length or provisioning rate. Provisioning rate did, however, predict song rate; individuals fed more frequently by their parents sang at a higher rate, especially small individuals and those born in small broods. These results support the developmental stress hypothesis and provide further evidence for the potentially far-reaching consequences of the conditions experienced during early life, with poorer conditions being associated with less complex song produced at a

lower rate. Song characteristics may therefore provide females with information regarding both the current condition and developmental history of an individual male.

## 5.2 Introduction

In many bird species, males produce complex songs to defend their territories and attract a mate, and female choice is thought to be a major driver of the evolution of large song repertoires (Catchpole & Slater 1995; Searcy & Yasukawa 1996). Song is expensive to produce, and individuals can also incur significant energetic costs developing the brain structures necessary for learning complex songs (Nowicki, W A Searcy, et al. 2002). Furthermore, time spent learning and performing song is time taken away from other essential activities such as foraging (Oberweger & Goller 2001) and increases exposure to predation (Gil & Gahr 2002). Females can therefore use song characteristics as measures of male quality (Searcy & Andersson 1986), which can result in benefits such as proficient paternal care, territory defence, foraging and predator avoidance, as well as better genes for their offspring (Catchpole & Slater 1995; Greig-Smith 1982; Nowicki et al. 2000).

Songbirds have a specialised auditory-vocal area of the forebrain that is responsible for song learning and production known as the "song system" (Gil & Gahr 2002; Nowicki et al. 1998). This system is made up of two interconnecting pathways, the higher vocal centre (HVC) and robust nucleus of the arcopallium (RA; Nowicki et al. 1998); it has been shown that damage to these two regions of the brain can be detrimental to adult song production (Nottebohm et al. 1976). The development of these pathways occurs during the nestling and fledgling period, a time when young birds are most vulnerable to stress (Nowicki et al. 1998; Nowicki et al. 2000). Song learning can therefore be an indicator of early ontogeny as individuals that have not

encountered stress are more likely to have the resources available to invest in optimal brain development (Nowicki et al. 1998). Adult birds may therefore be constrained in the quality of the song they are able to produce because the costs of stress experienced during development cannot be compensated for later in life (Buchanan & Catchpole 2000; Buchanan et al. 2003; Catchpole 1996; Doutrelant et al. 2000; Nowicki et al. 2000). Originally proposed as the 'nutritional stress hypothesis' (Nowicki et al. 1998), this idea is now known as the 'developmental stress hypothesis' to incorporate all of the different stressors young are exposed to in the early phases of life (Buchanan et al. 2003).

Many studies have found evidence to support the developmental stress hypothesis by using dietary manipulation or the administration of the stress hormone corticosterone, and testing for negative effects of these treatments on song characteristics in adulthood. Examples include: European starlings (*Sturnus vulgaris*; Buchanan et al. 2003; Spencer et al. 2004), zebra finches (*Taeniopygia guttata*; Brumm et al. 2009; Buchanan et al. 2004; Spencer et al. 2003), swamp sparrows (*Melospiza georgiana*; Nowicki et al. 2002) and song sparrows (*Melospiza melodia*; Schmidt et al. 2013). Most studies have involved captive birds, with very few being conducted on wild bird populations. Nowicki *et al.* (2000) found that the length of the innermost primary feathers of nestlings were positively correlated to adult repertoire size in great reed warblers (*Acrocephalus arundinaceus*); Doutrelant *et al.* (2000) found that adult tarsus length, a known correlate of nestling tarsus and reportedly a good measure of stress, was found to correlate with repertoire size in blue tits (*Cyanistes caeruleus*). However, these studies were based purely on measurements of body size and no other early life conditions were taken into account.

In this chapter, I provide one of the first tests of whether early life conditions predict adult song characteristics in a wild bird, the white-throated dipper (*Cinclus cinclus*). Dippers are riverine passerines which sing highly complex songs (Magoolagan et al., in review). In Chapter 3, unpaired males recorded singing early in the breeding season were assumed to be attempting to attract a mate and were found to use a larger number of unique syllables within their songs

and sing at a higher rate than paired males. These song features are likely to be favoured by potential mates because females are known to prefer exaggerated traits in males (Catchpole 1996; Nowicki, W. A. Searcy, et al. 2002). The aim of this study was to test the developmental stress hypothesis by investigating whether these key song characteristics, repertoire size and song rate, are associated with the conditions individuals experience during the nestling phase.

## 5.3 Methods

### *5.3.1 Study population*

Data used in this chapter was recorded between January 2014 and June 2016, for more information on the study site and field methods, see Chapter 2.

### *5.3.2 Song recording and analysis*

Adult songs from 18 males born within the study site were recorded opportunistically between January 2014 and July 2016; for details on song recording and analysis see Chapter 2. For every song recorded, the behavioural context was classified according to the paired status and reproductive stage of the focal individual; further details of this are described in Chapter 3.

### *5.3.3 Statistical analyses*

General or generalised linear models were used to test whether repertoire size (Gaussian error structure) and song rate (Poisson error structure) in adulthood were predicted by the conditions experienced during early life. These explanatory variables included natal brood size (as a measure of sibling competition), parental provisioning rate (as a measure of nutritional stress, whether mediated by parental investment or food availability) and body size when nine

days old (as an index of nestling condition). Context at the time of song recording was also fitted to control for previously shown effects (Chapter 3) and age was fitted to control for potential effects of age of the individual at the time of recording. 'Brood size' was recorded as the highest number of nestlings within the individual's natal nest at any time during the nestling period, then input into the models as a factor: 'small' or 'large' according to whether it was above or below the mean (= 4.39). 'Provisioning rate' was the average of provisioning rates from all three observational periods as described in the methods (Chapter 2). 'Tarsus' was the tarsus length and 'body mass' the body mass of nestlings when nine days old. 'Age' was measured as a factor at the time of song recording as either 'first-year' or 'adult'. 'Context' was also a factor taken from the time of recording as 'solo', 'pre-breeding' or 'breeding' as discussed in Chapter 2. Prior to analysis, all variables were centred and standardised to improve interpretation of main effects (Schielzeth 2010). Collinearity between explanatory variables was checked using pairwise scatterplots, correlation coefficients and variance inflation factors (Zuur et al. 2010). 'Body mass' was dropped from the analyses as it was highly correlated with tarsus length ($r = 0.9$); all other variables were considered because there was no collinearity between them ($r < 0.4$ in all cases) and variance inflation factors were $< 3$ (Zuur et al. 2010). These five explanatory variables along with interactions between all pairs of early life variables were included in the full model. All models were constructed in R, version 3.2.2 (R Core Team, 2015), and the full model for each song characteristic was subjected to the 'dredge' function in the package 'MuMIn' (Barton 2016) to rank all sub-models by Akaike's Information Criterion, with the Hurvich and Tsai correction for small sample size (AICc) (Burnham & Anderson 2002; Hurvich & Tsai 1989). If $\Delta AICc \leq 2$ in two or more of the most parsimonious models, model averaging was performed. Models were validated by plotting the distribution of the residuals, the residuals versus the fitted values and the residuals versus each of the covariates (Zuur et al. 2009).

## 5.4 Results

Repertoire size was best explained by null model, however, the averaged model showed a small effect of context in which an individual's songs were recorded; solo males used the highest number of unique syllables (Table 1). There was also a small effect of brood size, with birds born in smaller broods having slightly larger repertoires (Table 1, Figure 1). No other terms were retained in the best-fitting models (Appendix 2, Figure 1).

Provisioning rate was an important predictor of song rate; nestlings which were fed at a higher rate by their parents sang at a higher rate as adults. However, the best-fitting models also contained interactions between provisioning rate and both brood size and tarsus length; the increase in song rate with provisioning rate was greater for smaller individuals and birds in small broods (Table 2; Figures 2 and 3, respectively). Age and context were not retained in the best-fitting models (Appendix 2, Figure 2).

## 5.5 Discussion

The results show that the conditions experienced by individual dippers during early life predict the quality and quantity of song they produce as adults. Behavioural context was found to be the most important predictor of repertoire size, supporting previous findings that solo males use a larger number of unique syllables (Chapter 3), but repertoire size was also found to be larger in birds from small broods. This is the first evidence from wild birds of a relationship between sibling competition and song characteristics in adulthood, although similar findings have been reported in captive studies. Male zebra finches (*Taeniopygia guttata*) from larger

**Table 1.** The model selection table for the global model of a general linear model examining the factors associated with adult male repertoire size:

lm(repertoire ~ context + age + provisioning rate + brood size + tarsus length + provisioning rate * tarsus length + brood size * tarsus length)

| Model # | (Intercept) | Age | Brood size | Context | Provision rate | Tarsus length | Brood size x Tarsus length | Provision rate x Tarsus length | df | LogLik | AICc | Delta | Weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 69.83 | | | | | | | | 2 | -83.33 | 171.46 | 0.00 | 0.25 |
| **5** | 91.00 | | | + | | | | | 4 | -80.87 | 172.82 | 1.36 | 0.13 |
| **3** | 76.75 | | + | | | | | | 3 | -82.75 | 173.22 | 1.76 | 0.10 |

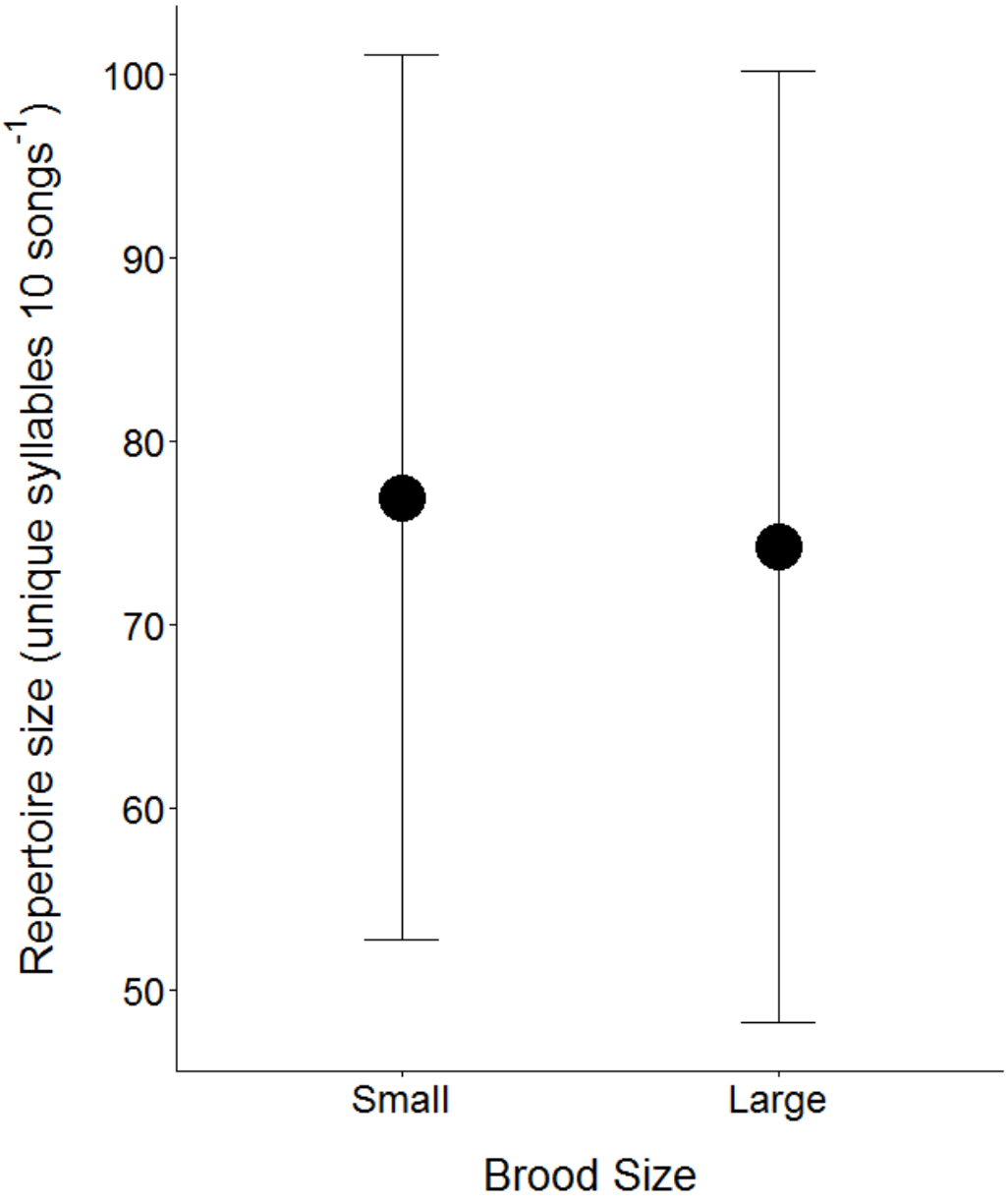


**Figure 1.** Adult repertoire size for nestlings in different sized broods (smaller or larger than the mean), predicted for solo males. Error bars show the 95% confidence intervals.

**Table 2.** The model selection table for the global model of a generalised linear model of the factors associated with adult male song rate:

glm(song rate ~ context + age + provisioning rate + brood size + tarsus length + brood*provisioning rate + brood size * tarsus length + tarsus length * provisioning rate)

| Model # | (Intercept) | Age | Brood | Context | Provision rate | Tarsus length | Brood size x Provision rate | Brood size x Tarsus length | Provision rate x Tarsus length | df | logLik | AICc | delta | weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **187** | 1.97 | | + | | 0.75 | 0.04 | + | | -0.30 | 6 | -60.50 | 140.63 | 0.00 | 0.43 |
| **43** | 2.03 | | + | | 0.90 | | + | | | 4 | -65.10 | 141.28 | 0.65 | 0.31 |

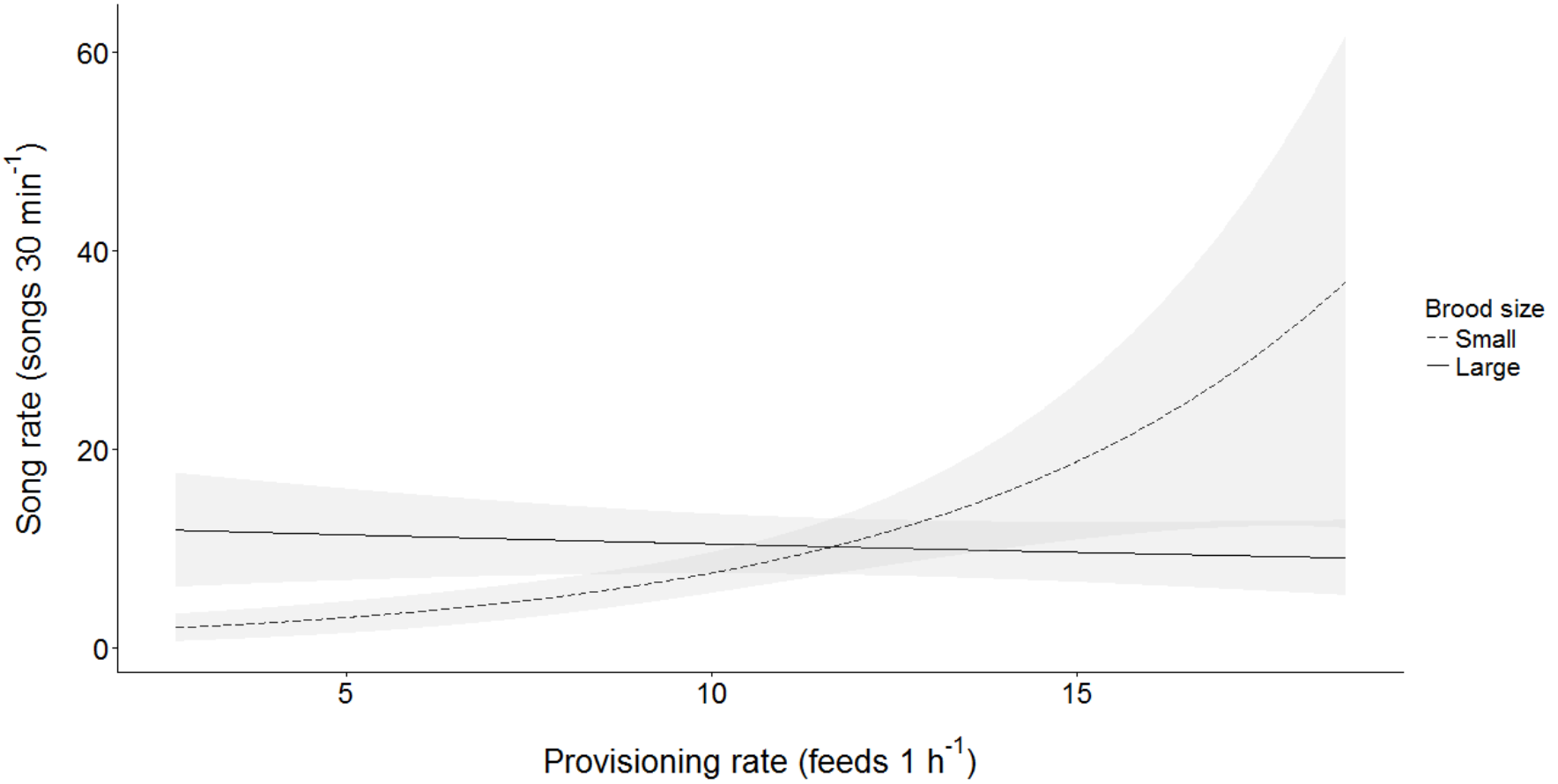


**Figure 2.** The relationship between the rate at which nestlings were provisioned and their song rate as adults, predicted for mean nestling tarsus length (25.56 mm). The *solid line* shows the relationship for small brood sizes (< 4.39 nestlings) and the *dashed line* shows the same relationship for nestlings in large broods (> 4.39 nestlings). The 95% confidence intervals are shown in grey.

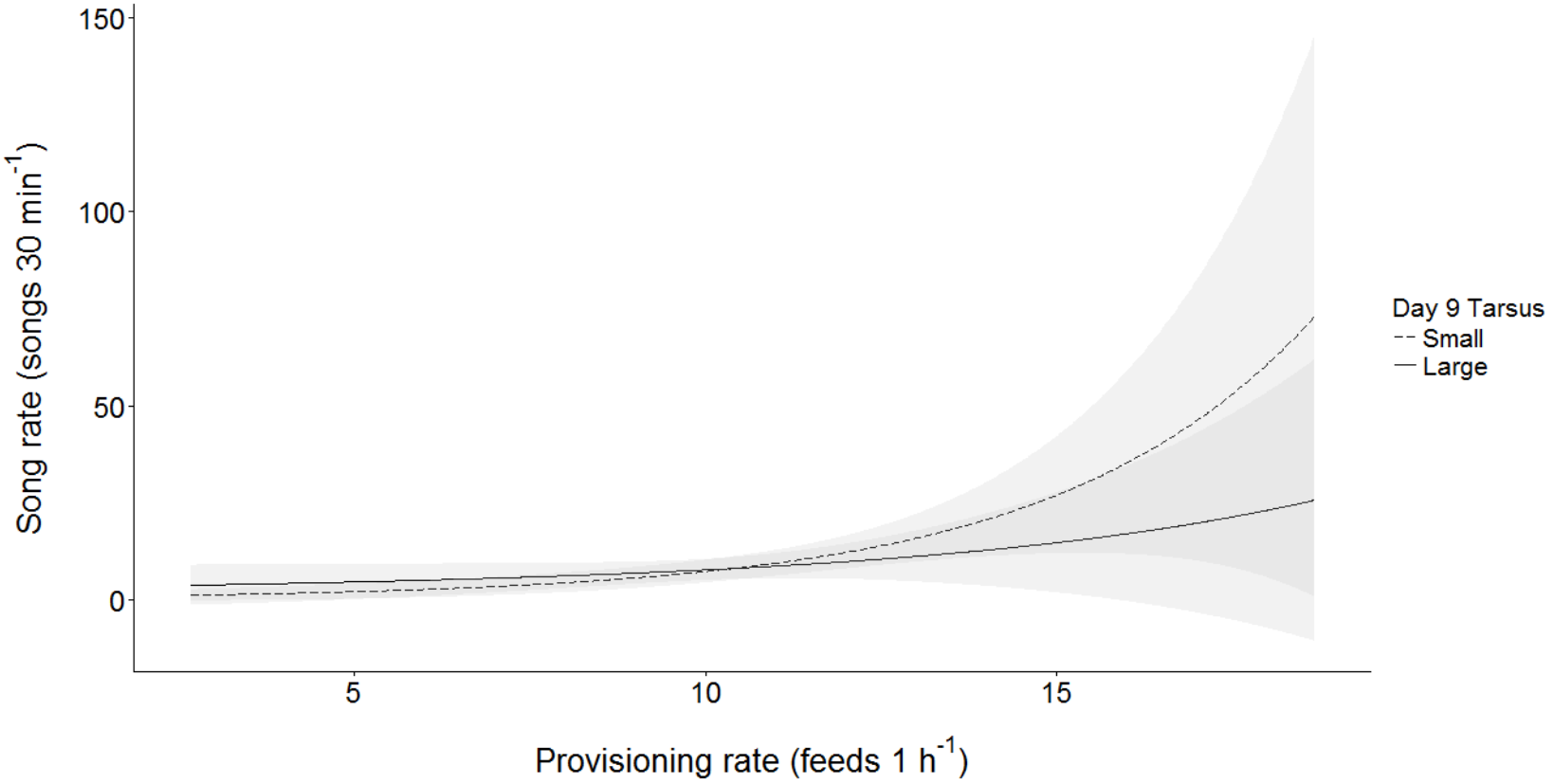


**Figure 3.** The relationship between the rate at which nestlings were provisioned and their song rate as adults, predicted for small broods (<4.39 nestlings). The *solid line* shows the relationship for nestlings with small tarsus length on day 9 (the shortest tarsus length measured on day 9 = 21.1 mm) and the *dashed line* shows the same relationship for nestlings with large tarsus length on day 9 (the longest tarsus length, measured on day 9 = 29.4 mm). The 95% confidence intervals are shown in grey.

broods had a reduced ability to learn the syntactical structure of their tutor's song (Holveck et al. 2008); Bengalese finches (*Lonchura striata domestica*) that came from larger broods had reduced phrase and syntactical complexity (Soma et al. 2006); and male blue tits (*Cyanistes caeruleus*) raised in experimentally enlarged broods were found to have shorter strophe bouts (Dreiss et al. 2006). The nestling phase is a critical period of development for birds, and larger brood sizes are likely to result in increased nutritional stress due to competition between siblings for the food provided by their parents. In many bird species, artificially increased brood sizes have consistently lower nestling weights and higher rates of mortality (Martin 1987). An increase in sibling competition is therefore likely to affect an individual's adult repertoire size because fewer resources are available to invest in these necessary brain structures during development (Nowicki et al. 1998). Smaller broods may also be indicative of lower parental quality, which in turn may impact on song development (Gilliland et al. 2016; Robinson et al. 2014), but this idea requires further investigation.

Early life conditions were also found to predict adult song rate. In contrast to repertoire size, song rate is considered a plastic characteristic usually associated with the current condition of an individual, rather than developmental history (Grunst & Grunst 2014; Møller et al. 1998; Schmidt et al. 2014). However, it is often reported that developmental stress can cause long-term damage and have a negative effect on adult phenotypes (Pfaff et al. 2007), and therefore affect an individual's ability to cope within their current environment (Macdougall-Shackleton et al. 2009). For example, zebra finch chicks that were raised on a poor quality diet accelerated their growth rate once resources had returned to normal, and exhibited increased resting metabolic rates as adults (Criscuolo et al. 2008). Increased resting metabolic rates cause an increase in the daily food demand (Criscuolo et al. 2008), which in turn may increase the time an individual spends foraging and decrease the time spent being vigilant. Individual song sparrows experiencing less stress during development were found to have better body condition as adults (Pfaff et al. 2007), and condition directly affects song output because only individuals with sufficient energy reserves are able to sustain high song rates (Grunst & Grunst 2014).

Decreases in parental effort can reduce nestling weight and increase nestling mortality (Buchanan & Catchpole 2000; Martin 1987), demonstrating that the amount of food parents are able to deliver has a strong effect on the number of successfully raised young. As with all altricial species, dipper chicks are completely dependent upon their parents for food for the duration of the nestling period (O'Connor 1984; Tyler & Ormerod 1994). An increase in foraging time and the associated decrease in foraging success has been shown to cause reductions in clutch size, brood size, egg mass, nestling mass, growth rates and survival rates (Ormerod & Tyler 1989; Ormerod et al. 1991; Vickery 1992). Provisioning rate is therefore likely to have a major effect on the growth and size of each individual, and this may be greater in smaller broods where the per capita effect of a change in food availability is higher. The results of this study shows that individuals raised in small broods which were fed at a higher rate sang at a higher rate as adults, presumably because the additional resources available to these birds as chicks allowed them to maintain better body condition later in life (Nowicki, W A Searcy, et al. 2002; Nowicki & Searcy 2004). The apparent absence of this effect in larger broods may be due to sibling competition offsetting the benefits of an increase in the rate of food provisioning.

The relationship between provisioning rate and song rate also varied with nestling tarsus length. Increases in provisioning rate had a stronger positive correlation with song rates in nestlings which had shorter tarsi, possibly because smaller birds require relatively more resources to be able to invest in brain development alongside achieving fledging mass at the same time as their siblings (Nowicki, W A Searcy, et al. 2002). This is the first study to provide evidence that early life conditions can influence song rate, but others have shown that nestling size correlates positively with adult song repertoires (Doutrelant et al. 2000; Nowicki et al. 2000) and adult condition positively correlates to song rates (Grunst & Grunst 2014). Nestling condition can constrain neural capacity within an individual and thus affect song development later in life (Nowicki et al. 2000). Therefore, individuals that have experienced lower stress will

be in better condition and possess the extra resources required to invest in song production (Nowicki et al. 2000).

## 5.1 Conclusion

Together, the results presented here suggest that males with the ability to produce songs of higher quality or at higher rates experienced better conditions during development, thus supporting the developmental stress hypothesis. This provides important evidence from a wild bird that there is a link between early life conditions and song characteristics in adulthood. It is possible that female dippers use song characteristics as a measure of male quality given that it may convey a male's condition and his developmental history. Song recorded from solo males actively seeking a mate early in the breeding season had the highest song rates and larger repertoires, (Chapter 3), indicating a likely role for female choice in selection for song rate and complexity. Further research is now needed in order to better understand the relationship between early life conditions and song quality. Analyses of song collected from males across all contexts would provide further insights into the impact on repertoire size; experimental manipulation of diet during the nestling period would be particularly useful for understanding the extent to which nutritional stress influences song characteristics.

# Chapter 6

# Song characteristics and reproductive success in adult males

## 6.1 Abstract

One function of complex vocal communication in songbirds is to advertise male quality. Elaborate song in many species provides an honest signal of male fitness correlates such as genetic quality, body condition, territory size or quality and parental care. Specific song characteristics such as complexity or song rate are often condition-dependent and should therefore correlate with reproductive success, but surprisingly few studies have shown this relationship in wild populations. Here, male song was recorded over three years from a marked population of dippers in order to test whether two key parameters, repertoire size and song rate, were associated with male condition and whether they predicted reproductive success. Neither song characteristic was correlated with age, but individuals with shorter wings had larger repertoires. Males with larger repertoires were also found to have a higher chance of fledging young and fledged more chicks per brood. Larger repertoires may enable smaller males to obtain and a better-quality territory or attract a higher quality mate. Song rate was greatest in unpaired individuals recorded early in the season, but these birds produced lighter chicks and had lower fledging success. This could be a reflection of inexperience, but may also be because these birds did not have a mate from the previous season which is known to delay reproduction, with possible consequences for breeding success. Overall, these results support the idea that song complexity indicates male quality and is therefore an important determinant of fitness.

Song rate may reflect current condition but contextual variation might mask correlations with reproductive success.

## 6.2 Introduction

The evolution of elaborate song in male birds is thought to be driven by a combination of intersexual selection and intrasexual competition (Catchpole 1980). In most passerines, song functions to advertise male quality in both mate attraction and territorial defence (Catchpole 1980; Kroodsma & Byers 1991), and variation in song complexity between males is widely thought to reflect differences in genetic quality (Nowicki et al. 1998; Nowicki, W A Searcy, et al. 2002). Female choice selects for the evolution of exaggerated or more ornamented traits in males due to the benefits females can gain by mating with the signaller, in terms of the genetic quality for their offspring (Hoelzer 1989), and the size of a male's vocal repertoire is therefore thought to be an important determinant of reproductive success (Lambrechts & Dhondt 1986; Nowicki & Searcy 2004; Schmidt et al. 2014).

Male song is a secondary sexual characteristic; time spent singing and the development and production of complex songs are energetically costly activities and therefore likely to be important signals of male quality (Gil & Slater 2000; Hofstad et al. 2002; Nowicki et al. 1998). Genetic quality or the ability to provide the resources necessary for successful reproduction, such as a good territory or high levels of parental care, can be honestly communicated (Dolby et al. 2005). Song characteristics which are known to correlate with male or territory quality, and therefore influence female choice, include: complexity (Catchpole 1986; Hasselquist et al. 1996; Lampe & Espmark 2003; Mountjoy & Lemon 1997; Yasukawa et al. 1980), duration (Byers et

al. 2016; Kempenaers et al. 1997), amplitude (Ritschard et al. 2010), dialect (Nowicki et al. 2000), and song rate (Eens et al. 1991).

Repertoire size, a widely used measure of song complexity, has been found to positively correlate with a number of measures of male quality (Catchpole & Slater 1995; Kipper et al. 2006; Mountjoy & Lemon 1996; Pfaff et al. 2007; Spencer et al. 2004) and also with good early life conditions which are essential for the development of the neural regions required to produce complex song (Nowicki et al. 2000). Song complexity has also been shown to correlate with reproductive success; males with larger repertoires have been shown to pair earlier (Catchpole 1990; Lampe & Espmark 1994), have longer territory tenure (Hiebert et al. 1989; Potvin et al. 2013), and greater annual and lifetime reproductive success (Hiebert et al. 1989; Potvin et al. 2013).

The rate at which individuals sing is another characteristic thought to reflect male quality. Song rate has been linked to food availability, for example, and so may signal male condition or territory quality to a female (Manica et al. 2014; Ritschard & Brumm 2012). Singing at a higher rate is costly due to the increase in metabolic expenditure or the reduced time spent undertaking other essential activities such as foraging (Grunst & Grunst 2014; Nowicki & Searcy 2004). Thus, only males in good condition are able to maintain a high rate of song production (Gil & Gahr 2002). Males with higher song rates are known to pair and mate earlier in pied flycatchers (*Ficedula hypoleuca,* Gottlander 1987) and snow buntings (*Plectrophenax nivalis,* Hofstad et al. 2002); are subject to fewer extra-pair fertilisations in barn swallows (*Hirundo rustica*); Møller & Thornhill 1998); and invest more in parental care in the stonechat (*Saxicola torquata,* Greig-Smith 1982) and grey catbird (*Dumetella carolinensis,* Dolby et al. 2005).

The ‘differential allocation hypothesis’ states that individuals which possess more attractive qualities may provide lower parental investment because they attract mates willing to invest heavily in their offspring (Burley 1986; Sheldon 2000). For example, in marsh warblers

(*Acrocephalus palustris*), females paired with males which sing more complex songs produce larger clutches (Darolová et al. 2012). Similarly, in canaries (*Serinus canaria*), females invest greater concentrations of testosterone in their eggs, which can be very costly, when exposed to attractive male song (Gil et al. 2004). Understanding the fitness consequences of individual variation in song characteristics is therefore an excellent model for unravelling the interplay between male quality and female choice, and the role vocal communication plays in determining the reproductive success of both sexes.

A relatively large number of laboratory studies have shown that males with bigger repertoires have greater success attracting a mate (Catchpole 1986; Eens et al. 1991; Searcy & Marler 1981). However, these experiments may show effects that are not necessarily biologically meaningful due to the isolation of subjects and the lack of sensory stimuli and social interactions usually encountered in the wild (Byers & Kroodsma 2009). Therefore, to gain further insight into how song characteristics affect reproductive success, more studies of wild populations are needed. In particular, research on song parameters other than repertoire size and measures of reproductive success beyond mate attraction are currently lacking.

Here I investigate whether two song characteristics, repertoire size and song rate, are associated with measures of male condition, including body size and age, and whether they predict reproductive success in a wild population of dippers (*Cinclus cinclus*). These two song characteristics have been shown to correlate with early life conditions (Chapter 5) and may play an important role in mate attraction (Chapter 3). I predict that (1) larger and older males have greater repertoire sizes and sing at a higher rate; and (2) males with larger repertoires and higher song rates have greater reproductive success.

## 6.3 Methods

### *6.3.1 Study population*

Data were collected from an individually marked population of 40-50 pairs of dippers in the River Lune catchment near Sedbergh, Cumbria, UK. Adults were caught in mist nets or in hand nets placed over the nest and the following morphological measurements were taken on capture: (1) wing length; (2) tarsus length; and (3) body mass. Individuals were aged and sexed at capture according to their wing moult and wing length, respectively (Andersson & Wester 1971; Svensson 1992). For more details of the study population and field methods, see Chapter 2.

### *6.3.2 Reproductive success*

Each year, nests were located either by checking traditional sites at least each once a week during January and February or by observing nest-building behaviour at new sites. Once nest lining was complete, nests were checked once every two or three days. In most cases the 'lay date' of the first egg in a clutch could be determined because dippers lay one egg per day and start incubation when the clutch is complete, so lay date could be back-calculated once an incomplete clutch of cold eggs had been recorded. For this analysis, the date of the first egg laid is converted into Julian date (January $1^{st}$ = 1). Nestlings were weighed when nine days old using electronic scales (± 0.1g). Nests were checked daily for fledging from day 20 of the nestling period, and the number of chicks to fledge was taken as the size of the brood on day 9 minus the number of dead chicks found in or near the nest after fledging. Observations show that the majority of nestling mortality occurs before day 9 (unpublished data).

### *6.3.3 Provisioning rate*

Offspring provisioning rates were measured as described in Chapter 2. For this study, provisioning rates were measured by simply counting the total number of feeds made by the

male during the hour. 'Average provisioning rate' was then calculated by summing the provisioning rates from each observation session and dividing by the number of sessions (3 in 84% of cases, 2 for the remaining 16%).

### *6.3.4 Territory quality*

To measure water quality and food abundance within each pair's territory, kick samples were collected from a primary feeding location during the final week of the nestling period or the first week after fledging, as described in Chapter 2. The measures used in this study were invertebrate mass, which gives an idea of food availability within each territory, and Average Score Per Taxa (ASPT), which provides a measure of water quality, based upon the invertebrate families found within the sample (Armitage et al. 1983). The altitude of the territory was also included because it is known to affect the timing and success of reproduction in dippers (Tyler & Ormerod 1994).

### *6.3.5 Song recording and analysis*

Songs from 38 males were recorded opportunistically between January 2014 and June 2016; for details of song recording and analysis methods, see Chapter 2. For every song recorded, the behavioural context was classified according to the paired status and reproductive stage of the focal individual; further details of this are described in Chapter 3.

### *6.3.6 Statistical analyses*

General or generalised linear models were used to test whether repertoire size (Gaussian error structure) or song rate (Poisson error structure) were correlated with male size and condition (N = 38). Explanatory variables included wing length, tarsus length, body mass and age. Age was a categorical measure, recorded as either 'first-year' or 'adult' (Svensson 1992) and, as such, adults were considered to be in better condition because they have undergone a complete moult and are better foragers (Yoerg 1994; Yoerg 1998). Context at the time of song recording was also fitted as a factor because of previously shown variation in structure between

songs produced in different behavioural contexts (Chapter 3). Prior to analysis, all variables were centred and standardised to improve interpretation of main effects (Schielzeth 2010). Collinearity between explanatory variables was checked using pairwise scatterplots, correlation coefficients and variance inflation factors (Zuur et al. 2010). Providing there was no collinearity between variables ($r < 0.4$ in all cases), and variance inflation factors were < 3 they were kept in the analysis (Zuur et al. 2010). These five explanatory variables were used in the models.

Linear models, generalised linear models and generalised linear mixed models were used to test the relationship between the two song characteristics and different components of reproductive success, controlling for male age, size and measures of territory quality. To avoid over fitting the models, only one measure of body size was included. Tarsus length was chosen to reflect male condition as it was found to be an important factor during song development (Chapter 5) and, unlike wing length and body mass, it varies little over the duration of an adult's lifespan (unpublished data). The exploratory variables were therefore: age and tarsus length (as measures of body condition), invertebrate mass (as a measure of food abundance within the territory), ASPT score (as a measure of water quality within the territory), altitude (a known correlate of lay date and reproductive success), repertoire size (as a measure of song complexity) and song rate (as a measure of song quantity). All measures of reproductive success were taken from the same year the song was recorded for each individual. Collinearity between these variables was checked as described above, and ASPT was dropped from these models as it was highly correlated with age ($r = 0.6$); age was prioritised as it has been previously found to correlate with measures of breeding success in dippers (Wilson 1996). All other variables were considered because correlations were low ($r < 0.4$) and variation inflation factors small (< 3) unless otherwise stated.

A linear model was used to test for a relationship between the explanatory variables and lay date ($N = 28$). A general linear mixed model was used for a similar analysis of mean 'nestling body mass' (Gaussian error structure; $N = 64$, N broods = 16). Nestling body mass was measured on day 9 for each nestling. This model contained an additional explanatory variable,

average provisioning rate (as a measure of parental care), with brood identity fitted as a random factor (N = 16); invertebrate mass was dropped from the model because of its high correlation with song rate (r = 0.7) and altitude because of its correlation with repertoire size (r = -0.5). Generalised linear models were used to test whether 'fledging success' (Binomial error structure) and the 'average number of chicks fledged per brood' (Poisson error structure) were predicted by the explanatory variables (N = 29 in both cases). Fledging success is a binary measure with success referring to those males who raised at least one chick to fledging. The average number of chicks fledged per brood was calculated by dividing the total number of chicks raised to fledging by the number of broods attempted in that year.

All models were constructed in R, version 3.2.2 (R Core Team, 2015). For each response variable, the full model was subjected to the 'dredge' function in the package 'MuMIn' (Barton 2016) to rank all sub-models by Akaike's Information Criterion, with the Hurvich and Tsai correction for small sample size (AICc) (Burnham & Anderson 2002; Hurvich & Tsai 1989). If $\Delta AICc \leq 2$ in two or more of the most parsimonious models, model averaging was performed. Models were validated by plotting the distribution of the residuals, the residuals versus the fitted values and the residuals versus each of the covariates (Zuur et al. 2009).

## 6.4 Results

### *6.4.1 Male condition and song characteristics*

Repertoire size was best explained by wing length of the individual; males with shorter wing lengths had the largest repertoires (Table 1, Figure 1). Adult males had slightly larger repertoires than first-years, although the effect size was small (Table 1, Figure 2). Finally, there

**Table 1.** The model selection table for the global model of an averaged linear model of the factors examining the relationship between male condition and repertoire size:

lm(repertoire size ~ age + tarsus length + body mass + wing length + context)

| # | (Intercept) | Age | Context | Tarsus length | Body mass | Wing length | df | logLik | AICc | delta |
|---|---|---|---|---|---|---|---|---|---|---|
| 19 | 62.54 | | + | | 0.19 | -6.92 | 5 | -156.12 | 324.12 | 0.00 |
| 17 | 63.66 | | | | 0.17 | -9.18 | 3 | -158.78 | 324.27 | 0.15 |
| 20 | 64.69 | + | + | | 0.11 | -7.50 | 6 | -155.26 | 325.23 | 1.11 |

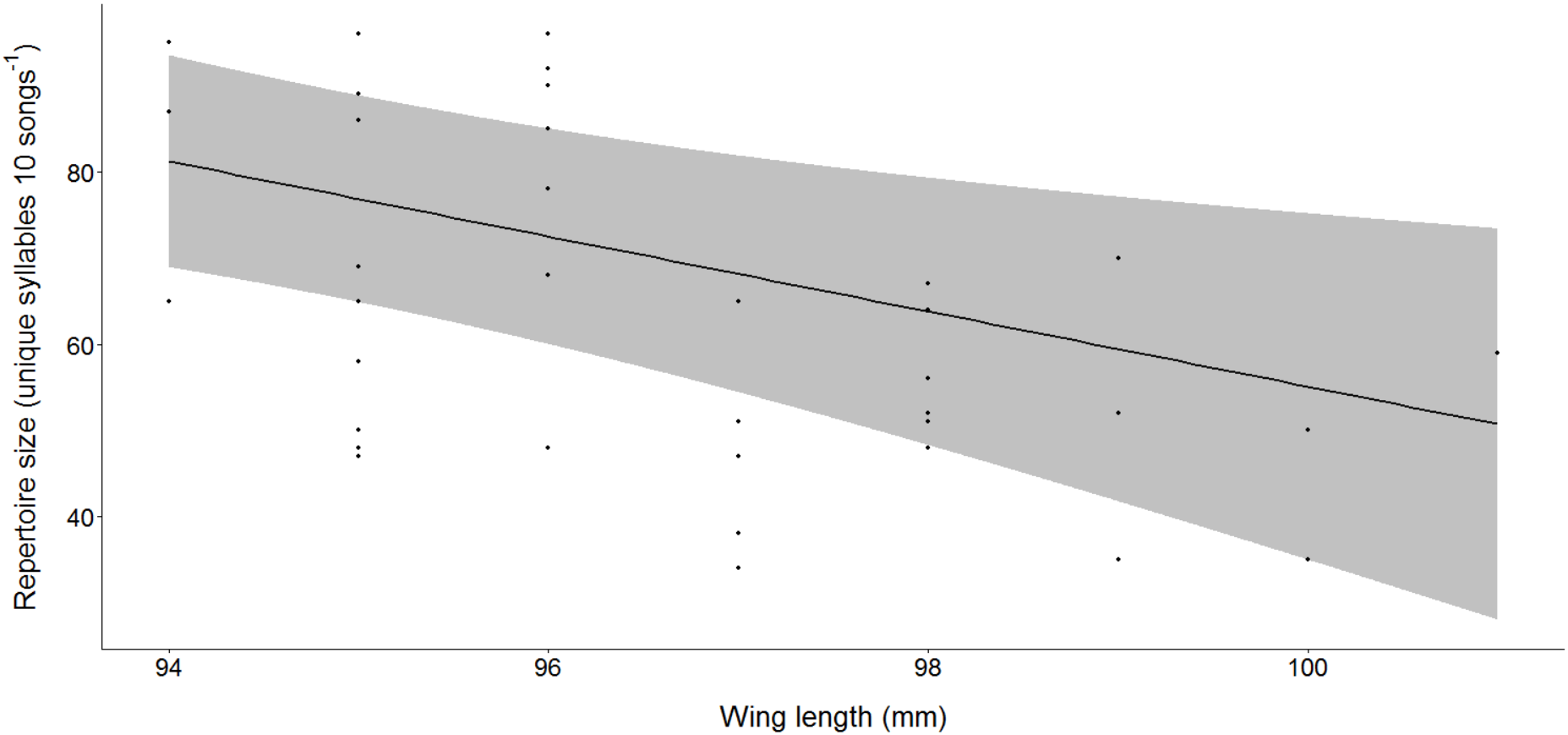


**Figure 1.** The relationship between repertoire size and wing length predicted for solo males in their first year. The 95% confidence intervals are shown in grey and points show the raw data.

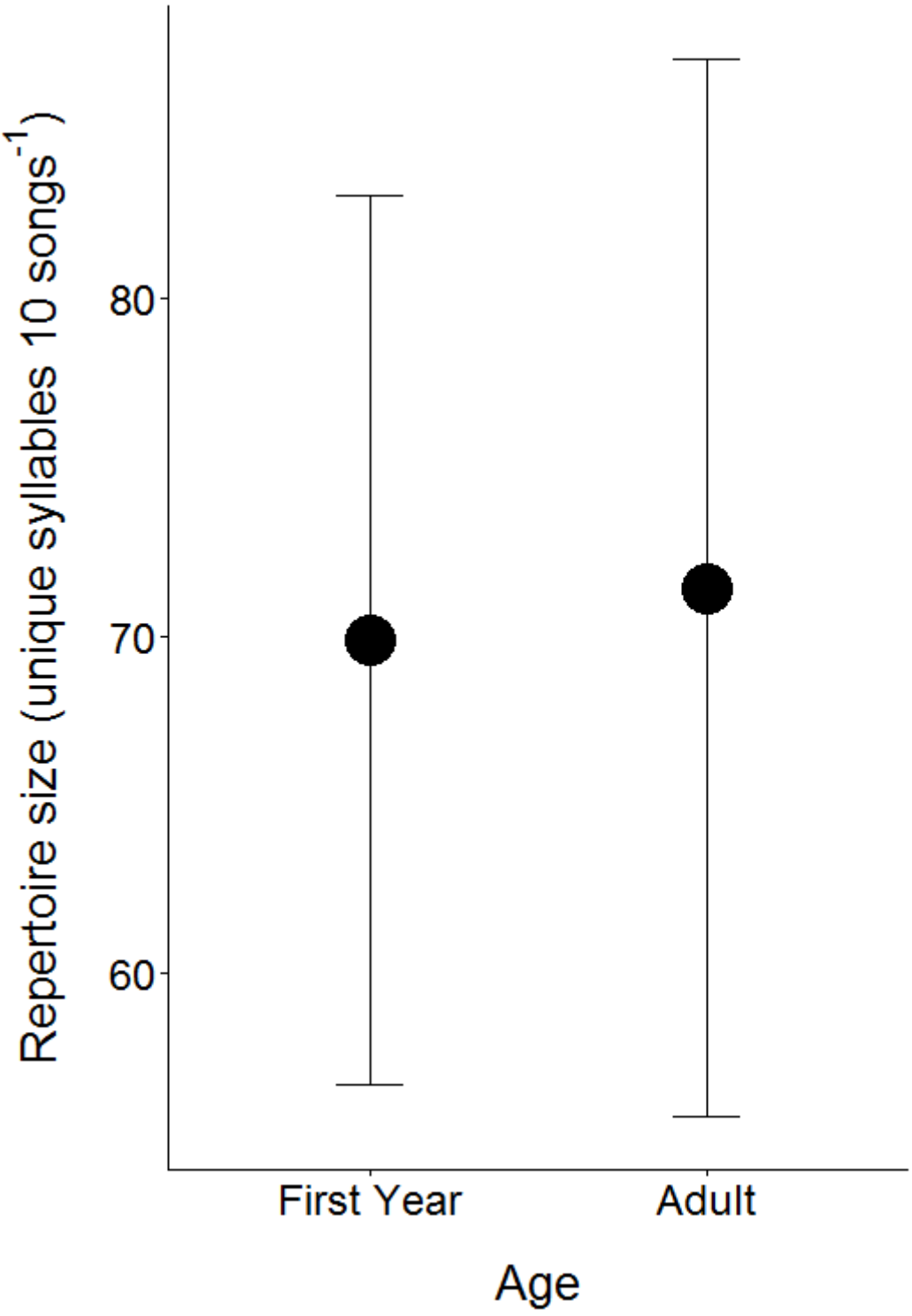


**Figure 2.** Repertoire size for males of different ages, predicted for solo males with average wing length (96.61 mm). Error bars show the 95% confidence intervals.

was a small effect of the context in which an individual's songs were recorded on repertoire size; solo males used more unique syllables within their songs (Table 1). No other terms remained in the best-fitting models (Appendix 3, Figure 1).

Song rate was best explained by the context in which an individual's songs were recorded; solo males were found to sing at a much higher rate (Table 2). Song rates were also found to be higher in first-years than in adults (Tale 2, Figure 3). Although wing length, tarsus length and body mass appeared in the best-fitting set of models, their effect sizes were very small and unlikely to be biologically meaningful (Table 2). No other terms were present in the best-fitting models (Appendix 3, Figure 2).

### *6.4.2 Song characteristics and reproductive success*

Lay date was best explained by the age of the male; adults had significantly earlier lay dates than first-years (Table 3, Figure 4). Altitude was found to be positively correlated with lay date (Table 3, Figure 5). Although tarsus length and invertebrate mass appeared in the best-fitting set of models, the effect sizes were extremely small and unlikely to biologically meaningful (Table 3). No other terms remained in the best-fitting models (Appendix 3, Figure 3).

Nestling mass was best predicted by song rate; males that sang songs at higher rates had lighter nestlings (Table 4, Figure 6). Nestling mass differed between males of difference ages, with adults having heavier nestlings than first-year males (Table 4, Figure 7), and both tarsus length and provisioning rate were positively correlated with nestling mass (Table 4, Figures 8 & 9, respectively), although the effect size was small in each case. Repertoire size appeared in the set of best-fitting models but had only a negligible effect size, and no other terms remained in the set (Appendix 3, Figure 4).

The best predictor of fledging success was repertoire size; males with larger repertoires had a greater probability of successfully fledging chicks (Table 5, Figure 10). Song rate also had an effect on fledging success; as song rate increased, an individual's predicted fledging success

**Table 2.** The model selection table for the global model of an averaged generalised linear model of the factors examining the relationship between male condition and song rate:

lm(song rate ~ context + wing length + body mass + age + tarsus length, family = "Poisson")

| # | (Intercept) | Age | Context | Tarsus length | Body mass | Wing length | df | logLik | AICc | delta | weight |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **8** | 2.06 | + | + | | 0.10 | | 5 | -165.66 | 343.2 | 0.00 | 0.14 |
| **11** | 2.11 | | + | 0.09 | | | 4 | -167.14 | 343.5 | 0.31 | 0.12 |
| **12** | 2.07 | + | + | 0.09 | | | 5 | -166.062 | 344.0 | 0.81 | 0.09 |
| **4** | 2.09 | + | + | | | | 4 | -167.423 | 344.1 | 0.87 | 0.09 |
| **3** | 2.15 | | + | | | | 3 | -168.782 | 344.3 | 1.08 | 0.08 |
| **7** | 2.13 | | + | 0.08 | | | 4 | -167.58 | 344.4 | 1.18 | 0.04 |
| **16** | 2.05 | + | + | 0.08 | 0.06 | | 6 | -164.97 | 344.6 | 1.46 | 0.04 |
| **15** | 2.11 | | + | 0.06 | 0.08 | | 5 | -166.48 | 344.8 | 1.64 | 0.03 |
| **20** | 2.07 | + | + | | | 0.07 | 5 | -166.53 | 344.9 | 1.75 | 0.03 |
| **24** | 2.05 | + | + | 0.09 | | 0.05 | 6 | -165.18 | 345.1 | 1.88 | 0.02 |

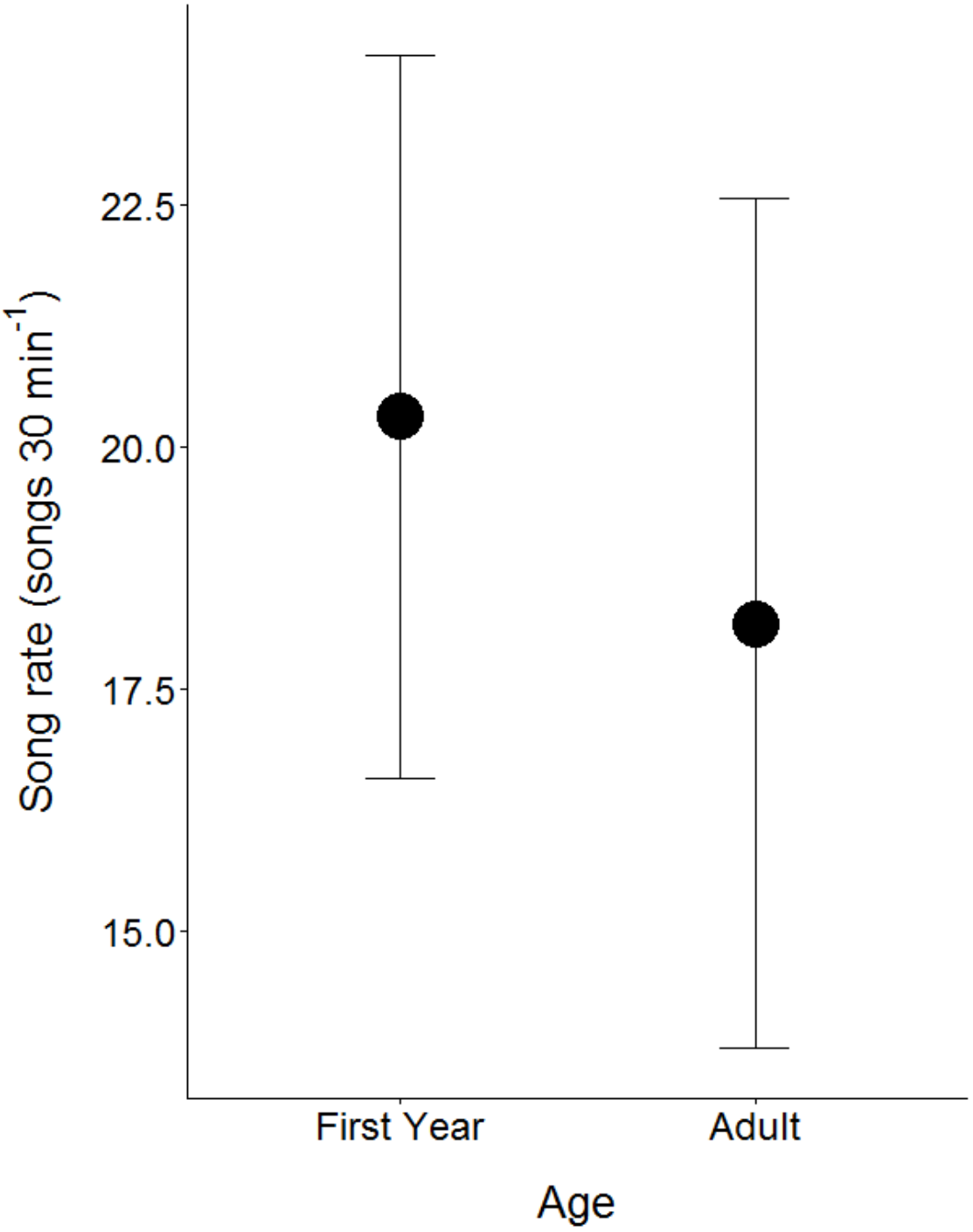


**Figure 3.** Song rate in males of different ages, predicted for solo males with average wing length (96.61 mm), tarsus length (29.94 mm) and body mass (66.40 g). Error bars show the 95% confidence intervals.

**Table 3.** The model selection table for the global model of an averaged linear model of the factors investigating the factors associated with lay date:

lm(lay date ~ repertoire size + song rate + age + tarsus length + invertebrate mass + altitude)

| # | (Intercept) | Age | Altitude | Invertebrate mass | Repertoire size | Song rate | Tarsus length | df | logLik | AICc | delta | weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 36 | 99.68 | + | 6.21 | | | | -3.25 | 5 | -100.18 | 213.10 | 0.00 | 0.23 |
| 4 | 99.57 | + | 6.05 | | | | | 4 | -101.96 | 213.67 | 0.57 | 0.18 |
| 40 | 99.12 | + | 6.83 | 2.51 | | | -3.22 | 6 | -99.12 | 214.24 | 1.15 | 0.13 |
| 8 | 99.00 | + | 6.68 | 2.56 | | | | 5 | -101.00 | 214.72 | 1.62 | 0.10 |

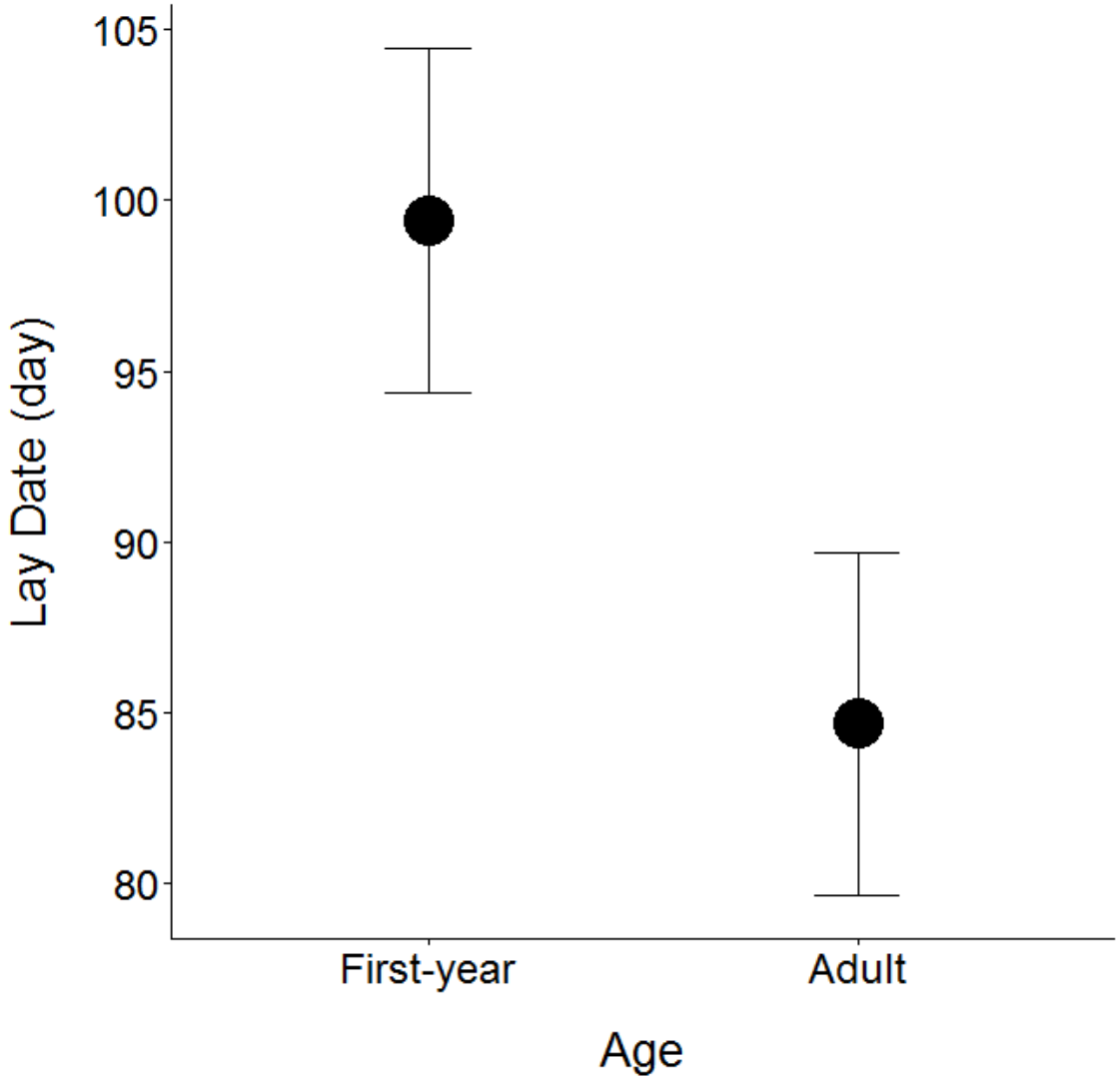


**Figure 4.** Lay date for males of different ages, predicted for males with average tarsus length (30.01 mm) and territories at average altitude (135.60 m), with average invertebrate mass (4.56 g). Error bars show the 95% confidence intervals. Lay date is measured using Julian date (January 1$^{st}$ = day 1).

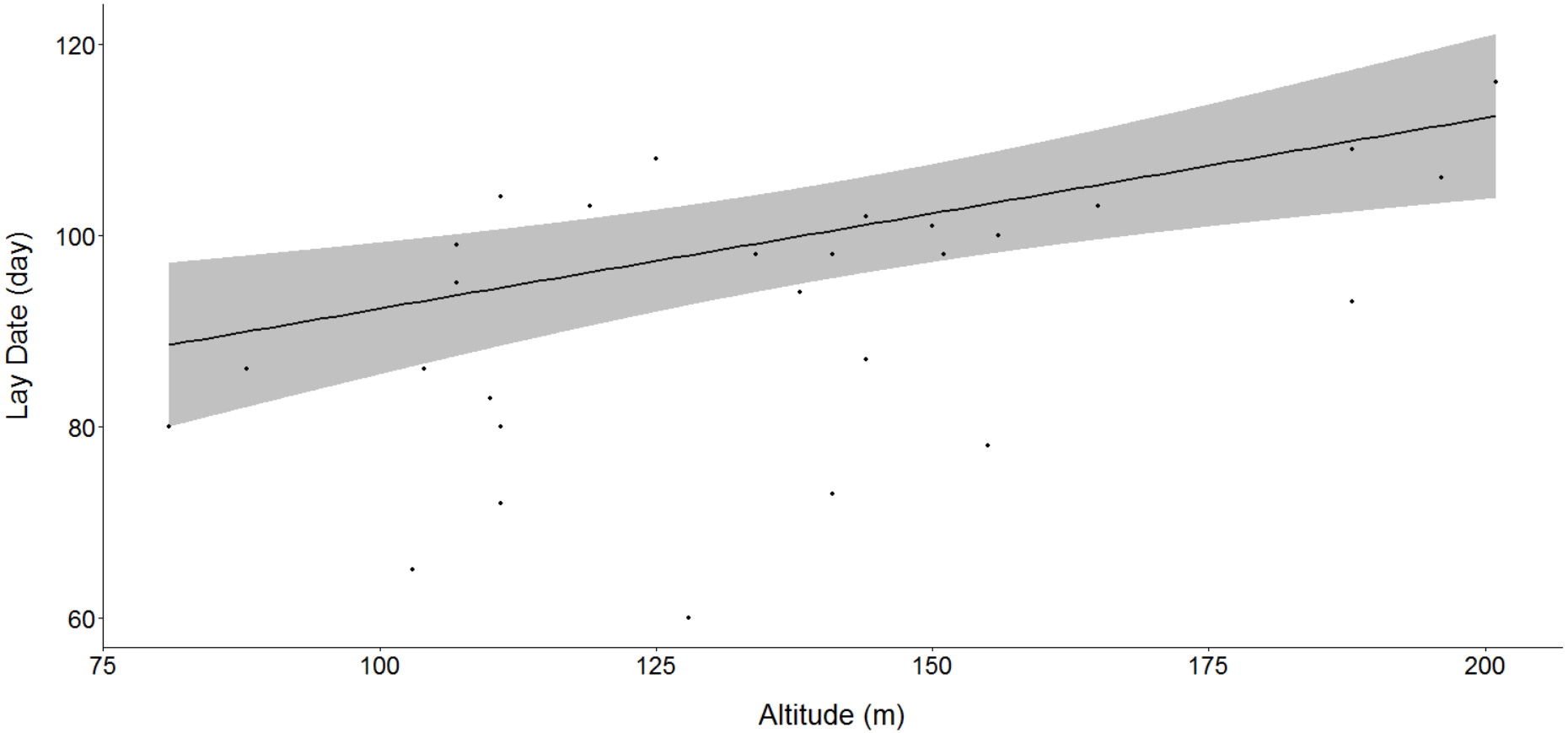


**Figure 5.** The relationship between lay date and altitude predicted for first-year males with average tarsus length (30.01 mm) and territories with average invertebrate mass (4.56 g). The 95% confidence intervals are shown in grey and the points show the raw data. Lay date is measured using Julian date (January 1$^{st}$ = day 1).

**Table 4.** The model selection table for the global model of an averaged generalised linear mixed model investigating the factors associated with nestling mass:

lmer(nestling mass ~ repertoire size + song rate + age + tarsus length + altitude + (1| brood identity))

| | (Intercept) | Age | Provision rate | Repertoire size | Song rate | Tarsus length | df | logLik | AICc | delta | weight |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **28** | 38.43 | + | 0.83 | | -2.04 | 1.40 | 7 | -174.70 | 365.40 | 0.00 | 0.08 |
| **32** | 38.48 | + | 0.83 | 0.14 | -2.06 | 1.43 | 8 | -173.40 | 365.43 | 0.03 | 0.08 |
| **30** | 38.36 | + | | 0.17 | -2.01 | 1.45 | 7 | -174.81 | 365.61 | 0.22 | 0.07 |
| **26** | 38.30 | + | | | -1.98 | 1.42 | 6 | -176.08 | 365.62 | 0.23 | 0.07 |
| **16** | 38.59 | + | 0.87 | -0.17 | -1.84 | | 7 | -175.18 | 366.36 | 0.97 | 0.05 |
| **12** | 38.66 | + | 0.87 | | -1.86 | | 6 | -176.46 | 366.40 | 1.00 | 0.05 |
| **14** | 38.46 | + | | -0.15 | -1.78 | | 6 | -176.61 | 366.69 | 1.29 | 0.04 |
| **10** | 38.53 | + | | | -1.80 | | 5 | -177.86 | 366.76 | 1.36 | 0.04 |
| **27** | 39.11 | | 0.87 | | -2.16 | 1.37 | 6 | -176.69 | 366.85 | 1.45 | 0.04 |
| **31** | 39.12 | | 0.86 | 0.29 | -2.19 | 1.43 | 7 | -175.45 | 366.91 | 1.51 | 0.04 |
| **25** | 39.06 | | | | -2.11 | 1.39 | 5 | -178.07 | 367.17 | 1.77 | 0.03 |
| **29** | 39.07 | | | 0.33 | -2.15 | 1.45 | 6 | -176.85 | 367.17 | 1.78 | 0.03 |
| **24** | 37.92 | + | 0.71 | -0.19 | | 1.16 | 7 | -175.66 | 367.32 | 1.92 | 0.03 |

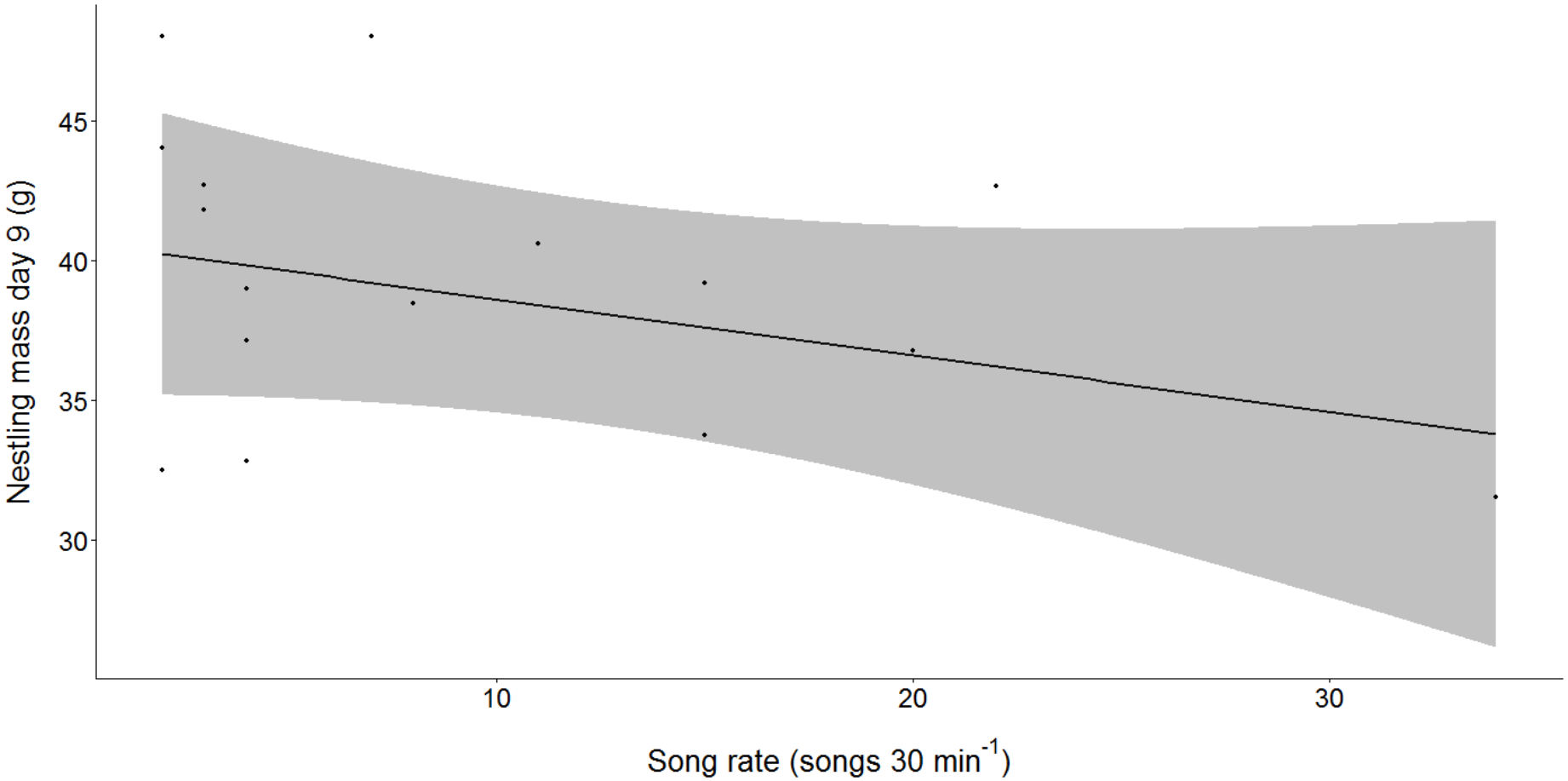


**Figure 6.** The relationship between nestling mass and male song rate predicted for first-year males with average tarsus length (29.94 mm), average provisioning rate (6.83 feeds $h^{-1}$) and average repertoire size (66.20 unique syllables). The 95% confidence intervals are shown in grey and the points represent the average nestling mass for each brood.

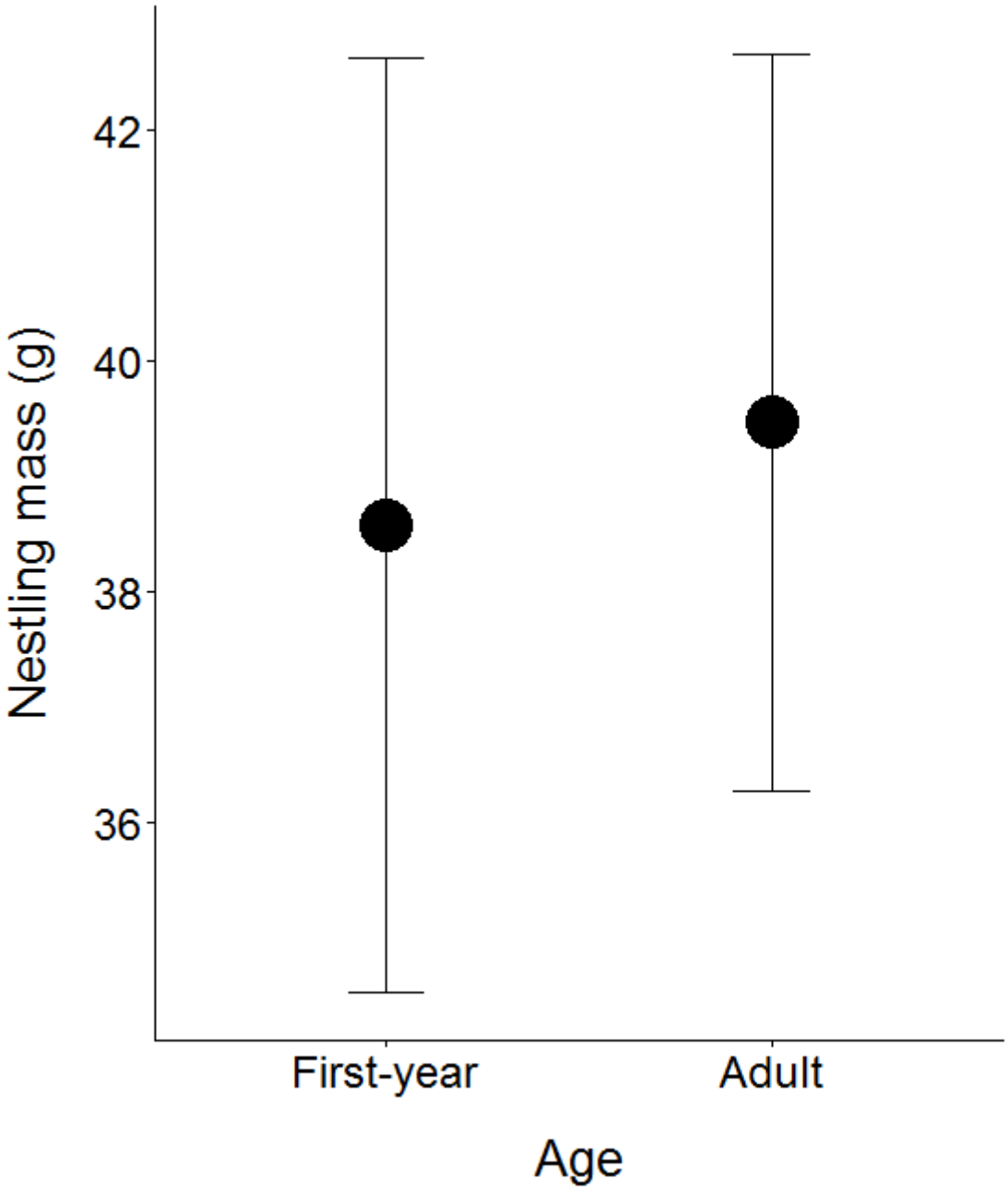


**Figure 7.** Nestling mass for males of different ages, predicted for males with average tarsus length (29.94 mm), average provisioning rate (6.83 feeds $h^{-1}$), average repertoire size (66.20 unique syllables) and average song rate (10.30 songs 30 $min^{-1}$). Error bars show the 95% confidence intervals.

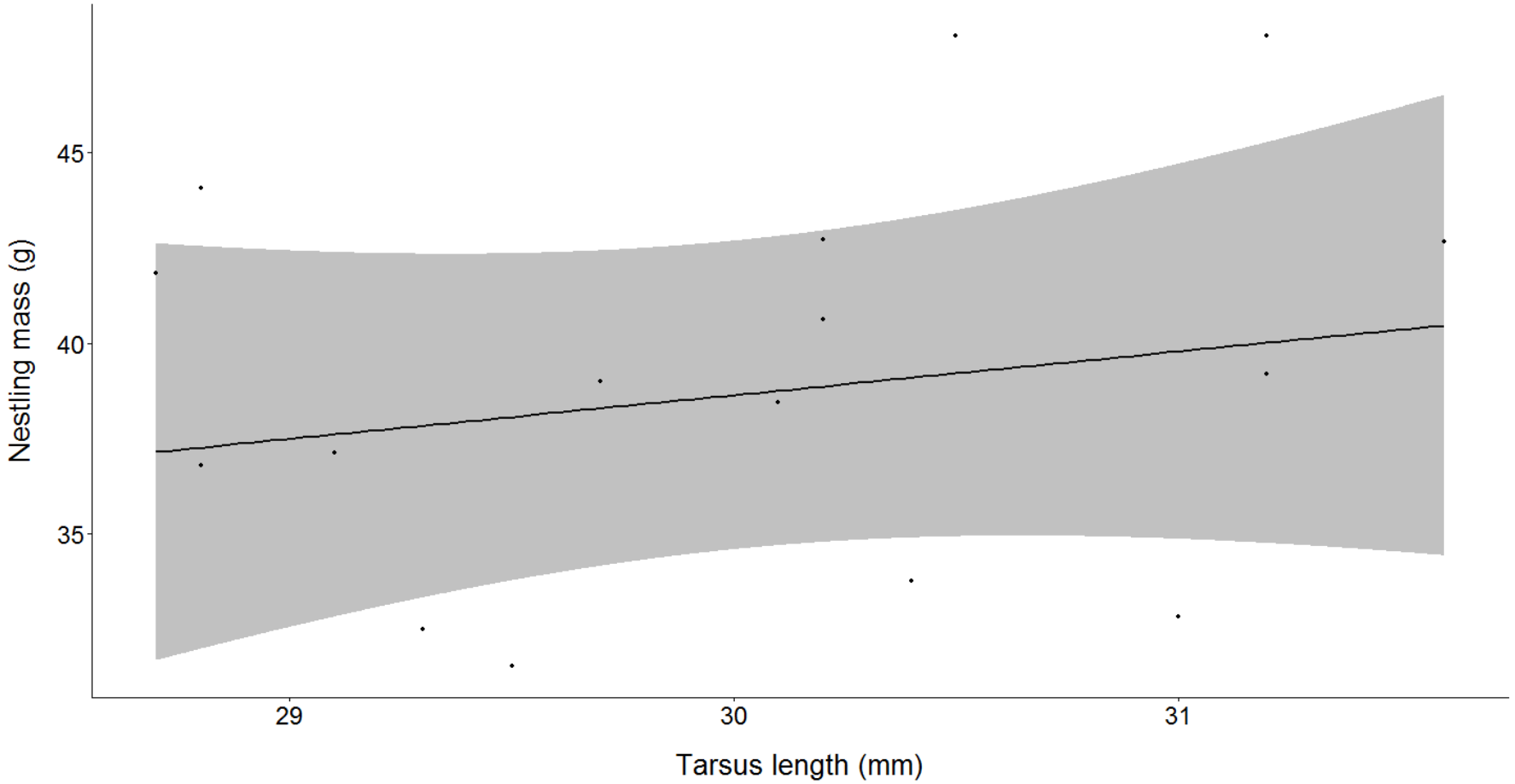


**Figure 8.** The relationship between nestling mass and adult tarsus length predicted for first-year males with average provisioning rate (6.83 feeds $h^{-1}$), average repertoire size (66.20 unique syllables) and average song rate (10.30 songs 30 $min^{-1}$). The 95% confidence intervals are shown in grey and points represent the average nestling mass for each brood.

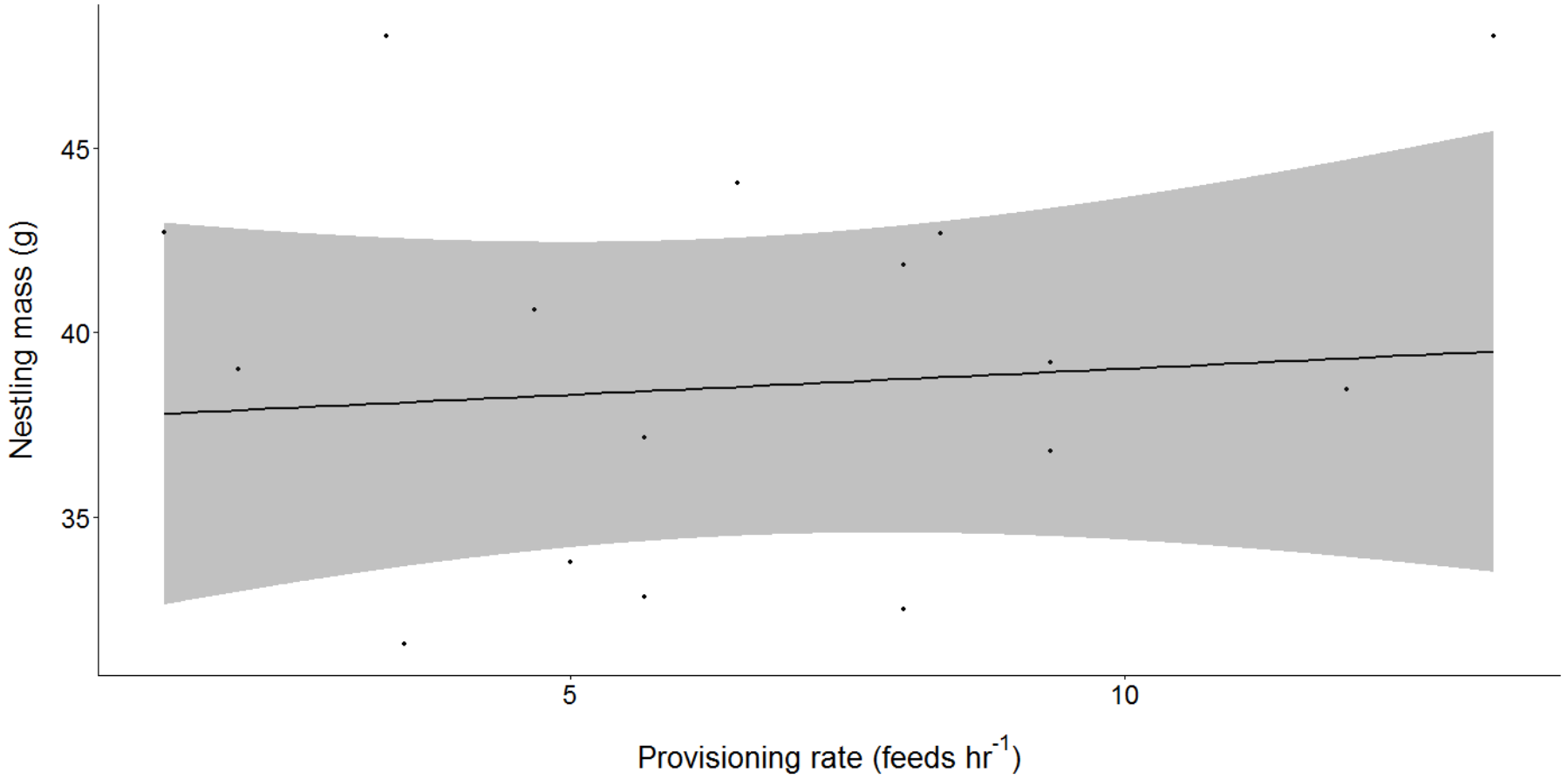


**Figure 9.** The relationship between nestling mass and male provisioning rate predicted for first-year males with average tarsus length (29.94 mm), average repertoire size (66.20 unique syllables) and average song rate (10.30 songs 30 $min^{-1}$). The 95% confidence intervals are shown in grey and points represent the average nestling mass for each brood.

**Table 5.** The model selection table for the global model of an averaged generalised linear model investigating the factors associated with fledging success:

glm( fledge success ~ repertoire + song rate + age + tarsus length + invertebrate mass + altitude, family = “Binomial”)

| # | (Intercept) | Age | Altitude | Invertebrate mass | Repertoire size | Song rate | Tarsus length | df | logLik | AICc | delta | weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.15 | | | | | | | 1 | -16.03 | 34.20 | 0.00 | 0.13 |
| 17 | 1.21 | | | | | -0.53 | | 2 | -15.25 | 34.97 | 0.76 | 0.09 |
| 9 | 1.23 | | | | 0.57 | | | 2 | -15.33 | 35.11 | 0.91 | 0.08 |
| 25 | 1.29 | | | | 0.58 | -0.54 | | 3 | -14.55 | 36.07 | 1.86 | 0.05 |

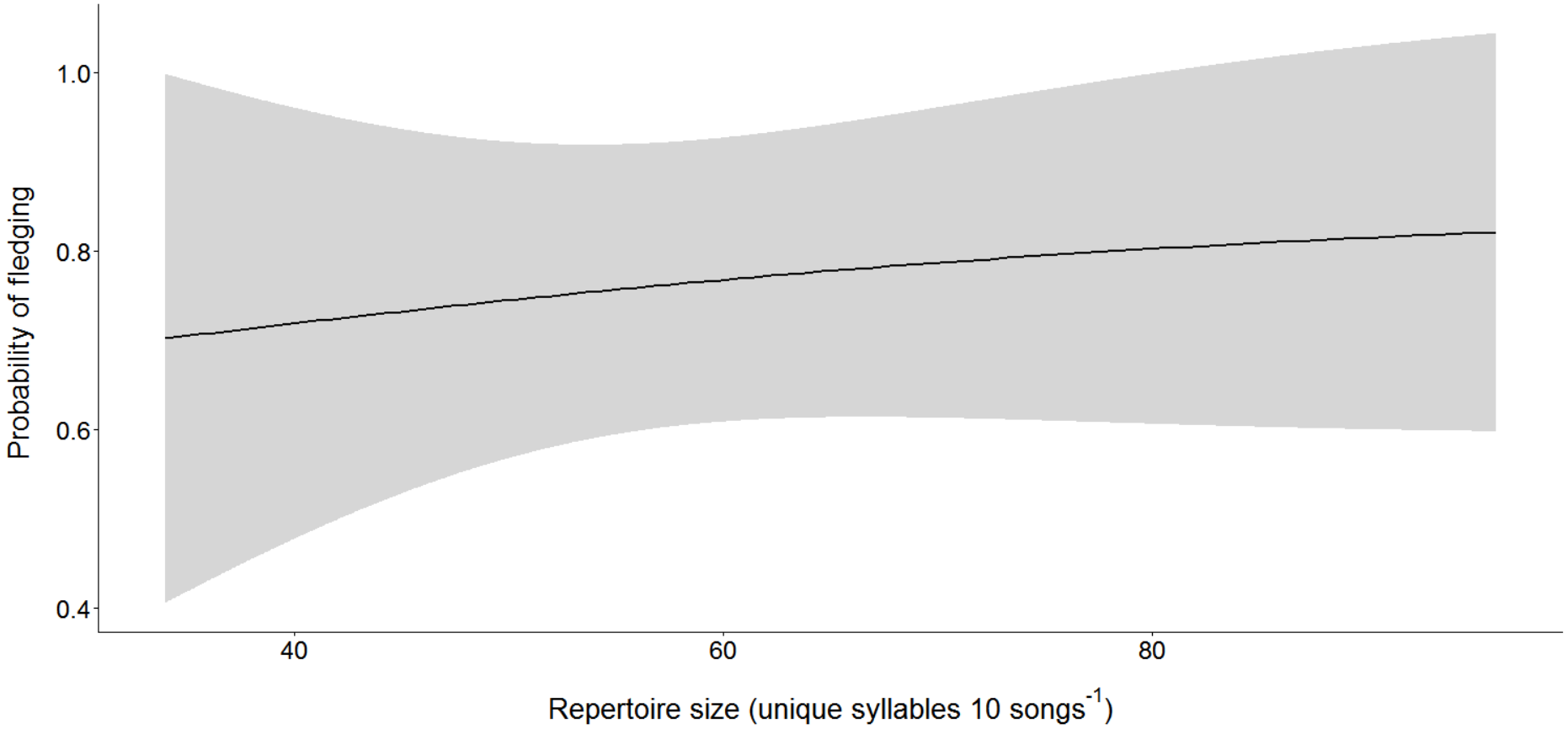


**Figure 10.** The relationship between fledging success and male repertoire size predicted for average song rate (10.97 songs $h^{-1}$). The 95% confidence intervals are shown in grey.

decreased (Table 5, Figure 11). No other terms remained in the best-fitting models (Appendix 3, Figure 5).

The average number of chicks fledged per brood was best explained by repertoire size; individuals with larger repertoires were found to fledge a higher number of chicks per brood (Table 6, Figure 12). Although the effect of invertebrate mass was small, territories with more invertebrates fledged a higher number of chicks per brood (Table 6, Figure 13). No other terms remained in the best-fitting models (Appendix 3, Figure 6).

## 6.5 Discussion

### *6.5.1 Male condition and song characteristics*

Wing length was found to be the best predictor of repertoire size; individuals with smaller wing lengths had larger repertoires. This opposes previous findings from studies of other species in which repertoire size positively correlates with measures of male quality or condition (Ballentine 2009; Dussord & Ritchison 2003; Hesler et al. 2012; Kagawa & Soma 2013; Kipper et al. 2006; Lampe & Espmark 1994; Lampe & Espmark 2003; Mountjoy & Lemon 1996). It is thought that the causes of shorter wing lengths in dippers might be constraints from undernourishment during the nestling period or foraging ability after fledging (Tyler & Ormerod 1994). Moreover, trade-offs during development mean that birds may have preferentially invested in the development of brain structures over wing length whilst in the nest (Nowicki et al. 1998), potentially compensating for any effects of small body size on reproductive success with the production of more attractive song. It is possible that larger birds may receive other benefits, such as higher survival rates, and therefore have more opportunities

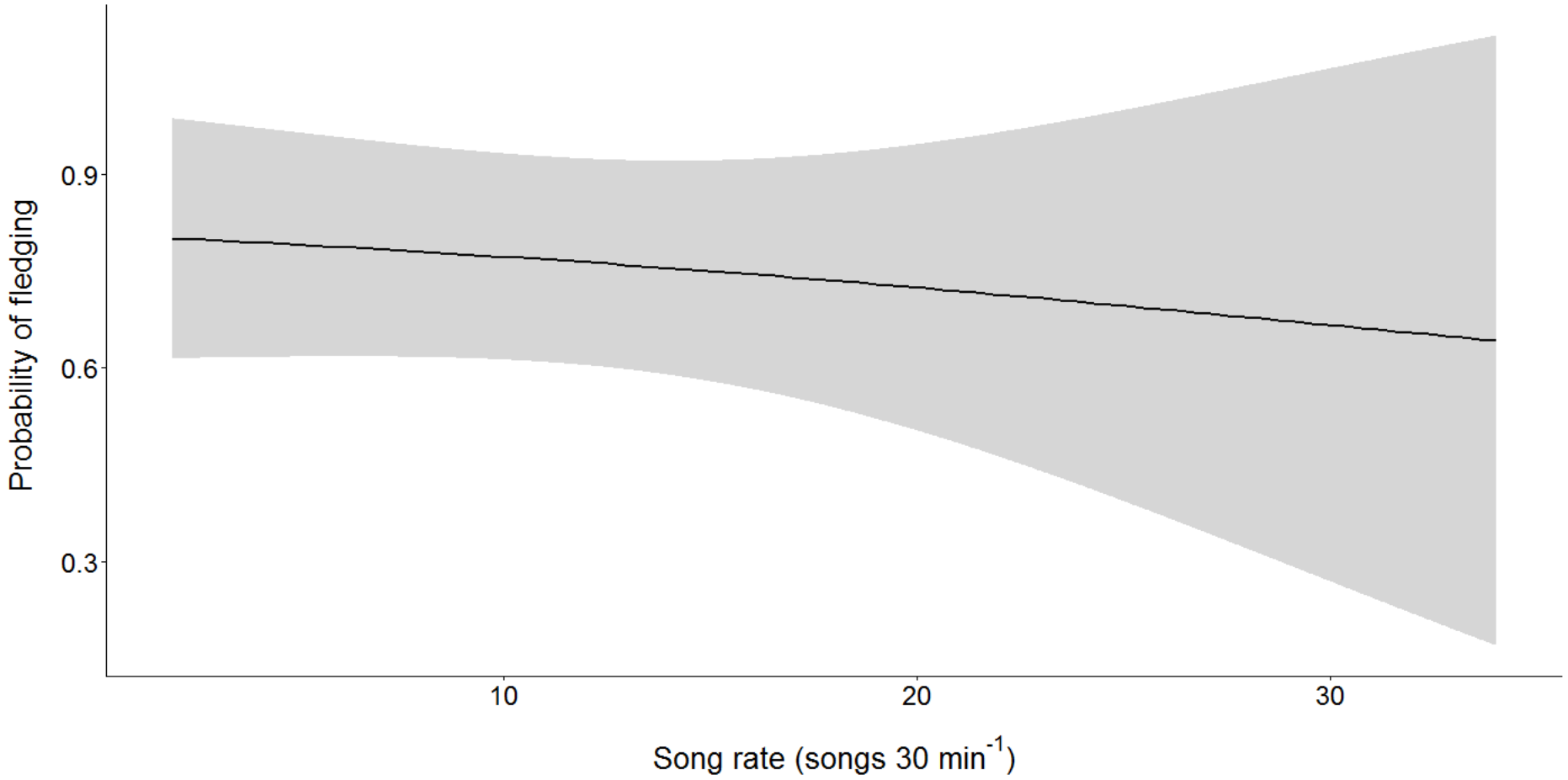


**Figure 11.** The relationship between fledging success and male song rate predicted for average repertoire size (60.41 unique syllables). The 95% confidence intervals are shown in grey.

**Table 6.** The model selection table for the global model of a linear model examining the factors associated with the average number of chicks fledged per brood:

lm(average chicks fledged per brood ~ repertoire size + song rate + age + tarsus length + altitude)

| Model # | (Intercept) | Age | Altitude | Invertebrate mass | Repertoire size | Song rate | Tarsus length | df | logLik | AICc | delta | weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **9** | 2.88 | | | | 0.68 | | | 3 | -52.10 | 111.20 | 0.00 | 0.18 |
| **13** | 2.88 | | | 0.41 | 0.63 | | | 4 | -50.95 | 111.60 | 0.40 | 0.14 |

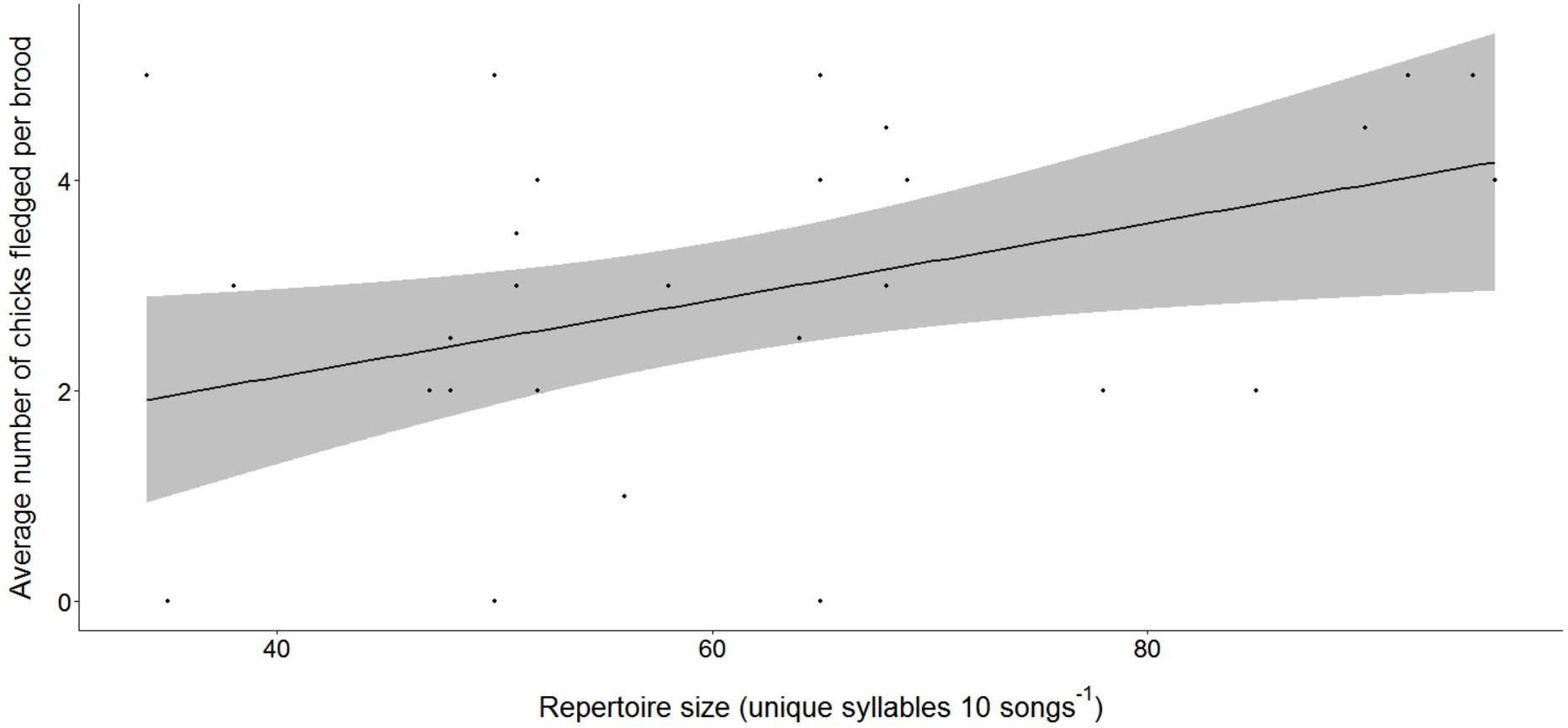


**Figure 12.** The relationship between the average number of chicks fledged per brood and male repertoire size predicted for average invertebrate mass (4.49 g). The 95% confidence intervals are shown in grey and the points show the raw data.

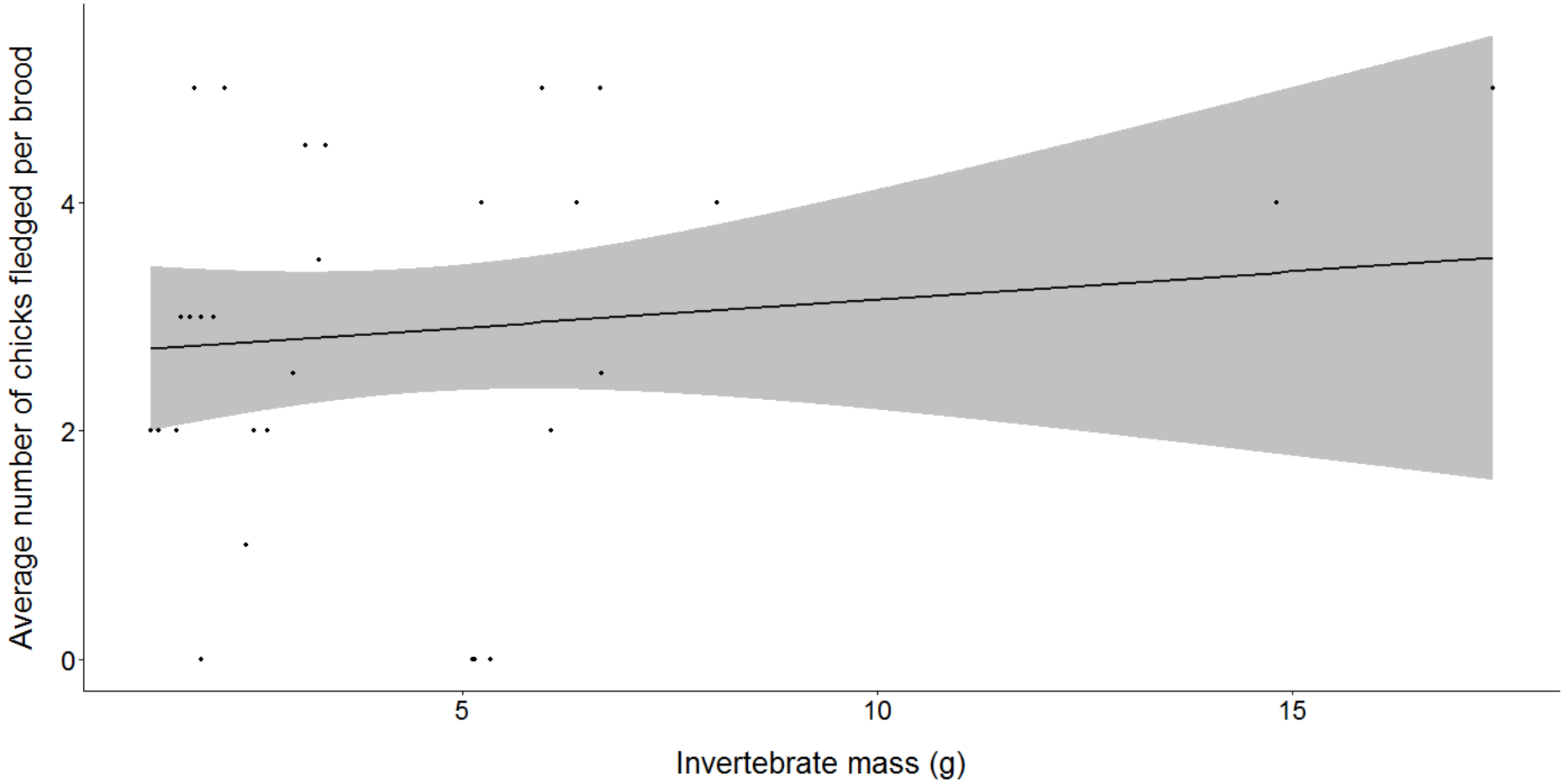


**Figure 13.** The relationship between the average number of chicks fledged per brood and territory invertebrate mass predicted for average repertoire size (60.41 unique syllables). The 95% confidence intervals are shown in grey and points show the raw data.

to reproduce and attain higher lifetime reproductive success. Smaller individuals could be actively maximising song effort in order to increase their chances of finding a mate. Further work is required to understand how song characteristics impact not just annual, but lifetime reproductive success, and to test whether smaller individuals are able to match the reproductive success of larger birds by using song characteristics to attract a mate more quickly or perhaps a mate of better quality.

Songs recorded from adult dippers showed slightly larger repertoires than those of first-year birds. Although it is not yet known how dippers learn their song, it seems likely they are open-ended learners due to the large size of their repertoires found during song analysis (Chapter 4), and this would explain the age effects reported here (Catchpole & Slater 1995; Nowicki et al. 1998). Age-specific variation in song complexity has been found in other species and has implications for reproductive success (Birkhead et al. 1997). However, insufficient longitudinal data have been collected from dippers to test how repertoire size changes with age.

Unsurprisingly, the context in which male songs were recorded was found to have an influence on repertoire size and song rate. Supporting findings in Chapter 3, solo males produced song at a higher rate using a larger number of unique syllables than song from other contexts. The effect of context was found to be much larger on song rate, which is to be expected considering song rate is a highly plastic song characteristic (Grunst & Grunst 2014). The majority of these solo males recorded are in their first breeding season and may need to actively defend their territories to a greater extent than males that have held a territory for longer; similar to the 'dear enemy' hypothesis, familiar neighbours behave less aggressively towards each other (Temeles 1994). This may also be the reason that song rates were higher in first-year than adult males.

### *6.5.2 Song characteristics and reproductive success*

This is the first study to investigate the relationship between song characteristics and reproductive success in dippers and the results suggest males with larger repertoires had greater

fledging success and fledged a larger number of chicks per brood. Given that males with larger repertoires were older and had shorter wings, these results may represent differential investment, as discussed previously. Males with larger repertoires may have the ability to attract higher quality females, and this could be the mechanism by which they improve their reproductive success (Burley 1986; Gil et al. 2004). Alternatively, repertoire size may be indicative of genetic quality or some aspect of male condition other than age or size (Macdougall-Shackleton et al. 2002).

Surprisingly, nestling mass and fledging success were found to be negatively correlated with song rate; this may be attributed to the greater number of solo males singing at higher rates. As these individuals were recorded as unpaired in January or February, they may be inexperienced or lower quality individuals that are yet to find a mate. Moreover, the fact that they are unpaired at the start of the year indicates that they will have bred later, and eggs hatched later in the season may miss the peak in food availability, causing lower chick masses and a higher incidence of nest failures (Tyler & Ormerod 1994).

Song rate is influenced by the condition of an individual and only males in good condition are generally able to achieve high song rates (Barnett & Briskie 2011; Møller & Thornhill 1998); this usually means that these males have access to a greater amount of food. It is conceivable that males use song to better defend territories (Catchpole & Slater 1995; Potvin et al. 2013) and females may then use these signals to assess not just the current condition and genetic quality of their prospective mate but also the quality of the territory they possess (Catchpole 1986; Howard 1974; Yasukawa et al. 1980). Song rate may still be a useful indicator of male quality and related to reproductive success, but the effects are currently being masked by the strong effect of context. Further work, recording song from individuals across the breeding season (i.e. in different contexts) would help to obtain a closer approximation of 'true' repertoire size and to better understand the relationship between song rate and breeding success.

Males with longer tarsi reared heavier nestlings; this could be due to heritability of male quality (Hasselquist et al. 1996; Kempenaers et al. 1997; Searcy 1992), or males in better condition being able to contribute more to raising a brood. As expected, males with high provisioning rates had heavier nestlings; parental care is extremely important in altricial species as this is the offspring's only source of food (Buchanan & Catchpole 2000; O'Connor 1984).

Whilst neither song measure was correlated with lay date, age was found to be the best predictor with adults having significantly earlier lay dates than first-years. Adults were also found to produce heavier nestlings. This supports previous findings from a study in Scotland which found that adult dippers had higher breeding success than that of first-years (Wilson 1996). Dipper pairs defend territories together year-round (Crowther *et al.*, in review), and older individuals that have bred together during a previous breeding season may stay together; this not only means they are more experienced at breeding but also that they will potentially be ready to begin nest-building earlier. First-year males have no prior breeding experience, and almost half of them mated with a first-year female (= 43%, N = 14), so the first brood of chicks may be smaller or less successful due to this inexperience. Dippers can have two broods in one breeding season and this is more likely to happen if they begin breeding in March (Tyler & Ormerod 1994). Over the three-year study, out of 23 broods that had laid their first egg in March, both members of the pair were adults in 61% of pairs and 52% of them had bred together in a previous year.

Altitude was found to positively correlate with lay date, with nests at lower altitudes laying earlier. This supports previous findings; altitude has been shown to delay laying dates in dippers because prey availability, specifically of those invertebrates which are calcium-rich, is more limited with increasing altitude (Tyler & Ormerod 1994). Aquatic invertebrates are essential to rearing a brood, and breeding abundance has been found to strongly correlate with the abundance of large aquatic invertebrates (Tyler & Ormerod 1994). Territories with heavier invertebrate masses were also found to fledge more chicks per brood. Despite this effect being small, the results are intuitive; territories with more food readily available are able to fledge a

greater number of chicks successfully. Therefore, males that hold low altitude territories that contain more invertebrates will have an advantage when it comes to breeding. It is notable that lay dates in dippers are not a straightforward indicator of reproductive success; Tyler and Ormerod (1994) found fledgling survival was highest in broods in the peak period of hatching in late April. It is therefore suggested that there is a trade-off between investing in early breeding and increasing their chances of rearing two broods in one season, or only having one brood at a time when prey items peak in abundance and offspring have the highest rate of survival (Tyler & Ormerod 1994).

## 6.6 Conclusion

Overall, the data show that song characteristics are correlated with measures of fitness in males. Males with larger repertoires had greater fledging success and were able to fledge a larger number of chicks per brood. It is possible these individuals had inherited genes which prepared them for stressful events during development, enabling greater investment in the necessary brain structures (Buchanan et al. 2003; Nowicki et al. 1998) or attracted a female willing to increase her level of parental investment in return for better genes for her offspring (Burley 1986). Further work recording song from the same birds across multiple contexts would help to reduce the potential masking effect of context on measures of male condition or reproductive success.

# Chapter 7

# Dietary manipulation experiment and growth rates of nestlings

## 7.1 Abstract

Poor conditions experienced during early life can significantly reduce the rate of growth and constrain development in young animals. If conditions improve, so-called 'catch up' growth allows individuals that have experienced developmental stress to subsequently accelerate growth and reach maturity within a similar time frame to those which have not. However, accelerated growth may carry significant fitness costs beyond the growth period, such as the production of lower quality song in birds. Here, the diet of wild dipper nestlings was experimentally manipulated in order to investigate (1) the impact of nutritional stress on growth rate and (2) whether nestling growth rate predicts adult song characteristics. Nestlings in experimental broods were measured and hand fed either a high or low quality diet every alternate day from the age of five days to 13 days; birds in control broods were removed from the nest and measured but returned without feeding. For body mass, tarsus length and wing length, linear mixed models were used to test whether initial body size and its interaction with diet quality impacted growth rates, controlling for other early life conditions such as brood size, parental care and territory quality. However, initial size was the only significant predictor of an individual's growth rate and this relationship did not vary with diet quality. Smaller nestlings allocated resources towards tarsus growth and body mass, whereas larger individuals invested in wing growth. Nestling growth rates were found to correlate with adult song characteristics; growth in body mass correlated positively with song rate and wing growth correlated negatively

with repertoire size. These results show that during the first few days of life, environmental conditions and maternal investment may have the greatest impact on an individual's growth trajectory, which in turn is associated with the amount and quality of song produced in adulthood. It is possible that dippers invest in either body size which facilitates singing at a higher rate, or in the development of the brain structures required for song learning, but rarely have the resources required to invest in both.

## 7.2 Introduction

The environmental conditions experienced during development can have adverse effects later in life (Lee et al. 2012b; Metcalfe & Monaghan 2001). Exposure to stress in early life can cause varying responses due to life-history trade-offs, with resources utilised depending upon what will maximise an individual's fitness at that time (Hegyi & Török 2007; Metcalfe & Monaghan 2001; Nilsson & Svensson 1996). During this developmental period, individuals may be exposed to a number of stressors within their environment including: unpredictable or low quality food supply, high parasite load, poor parental care and adverse weather conditions (Hegyi & Török 2007; Lepczyk & Karasov 2000; Mainwaring et al. 2010). These factors have been shown to impact adult body size and condition (Kogel & De Kogel 1997), immunocompetence (Lochmiller et al. 1993), reproductive behaviour and performance (Clark & Galef 1995; Lee et al. 2012b) and longevity (Birkhead et al. 1999; Metcalfe & Monaghan 2003).

Adverse developmental conditions, such as a decrease in food availability, are known to slow growth rates in juveniles (Hegyi & Török 2007). If conditions begin to improve, developmental plasticity enables individuals to accelerate their rate of growth in order to reach

their adult size before maturation (Lee et al. 2012b; Lepczyk & Karasov 2000; Metcalfe & Monaghan 2001). This increase in growth rate is known as catch-up or compensatory growth; if an individual instead prolongs the time taken to reach maturity it is known as parallel growth (Hegyi & Török 2007; Metcalfe & Monaghan 2001). Catch-up growth can have long-term deleterious effects and has been linked to impaired adult performance and a decrease in longevity and fecundity (Aldredge 2016; Bize et al. 2006; Konarzewski et al. 1996; Lee et al. 2012b; Metcalfe & Monaghan 2003; Stier et al. 2015). In a study of three-spined sticklebacks (*Gasterosteus aculeatus*), for example, exposure to relatively cold or warm water was used to change the normal growth trajectories of juveniles (Lee et al. 2012a). Once temperatures were returned to normal, catch-up or slowed-down growth was exhibited, with all individuals reaching the same average adult size. Individuals which increased their growth rates (catch-up growth) had on average a 14.5% reduction in lifespan, and these deleterious effects were greater in individuals that were given a shorter time period in which to compensate for the growth lost by the disturbance (Lee et al. 2012a). The plasticity seen in growth rates shows that they are optimal not maximal, and a fine balance between growth rate and fitness costs is favoured by selection (Bize et al. 2006; Metcalfe & Monaghan 2001; Metcalfe & Monaghan 2003).

Within altricial birds, the hatching order of chicks has a major influence on body size and therefore dominance within a brood, with last hatched nestlings often being smaller and at a competitive disadvantage to their siblings (Magrath 1990; Mainwaring et al. 2010). Even where there is a relatively high degree of synchrony in hatching, size asymmetries may exist within the brood due to genetic or maternal effects (Reed et al. 2009). It is common for parental feeding effort at the nest to decline or cease once the first chick has fledged (Nilsson & Svensson 1996), so nestlings usually fledge at a similar time to avoid being left behind. Catch-up growth strategies are therefore common within broods as smaller chicks must accelerate their growth rates to reach a size that will increase their chances of post-fledging survival (Nilsson & Svensson 1996). Other benefits to increasing growth rate and attaining a larger size in the nest include a higher capacity to obtain food as a nestling due to the competitive edge gained over

siblings and higher reproductive success later in life (Arendt 1997; Mainwaring et al. 2010; Richner 1992). However, catch-up growth may only be possible if conditions allow and, as stated above, there is increasing evidence that accelerated growth rates can cause a reduction in lifespan (Metcalfe & Monaghan 2003). It is believed that this negative correlation is due to high rates of cell division and oxidative stress, with rapid growth as the probable cause of increased levels of cell damage (Lee et al. 2012a); whilst a lower level of molecular damage occurs when more time is allowed for investment in growth (Metcalfe & Monaghan 2003). For example, growing structures such as flight feathers quickly may enable individuals to fledge on time and increase short-term survival, but these feathers may be less resistant to wear and break easily as a result, thereby decreasing fitness in the long-term (Dawson et al. 2000).

Different phenotypic traits are often under different selection pressures, so trade-offs can occur during the allocation of resources to features that are important for survival and fitness at different times (Birkhead et al. 1999; Bize et al. 2006; Mainwaring et al. 2010). This plasticity enables individuals to grow and develop to their best potential, under current conditions. For example, individual blue tit (*Cyanistes caeruleus*) nestlings have been shown to preferentially invest in tarsus growth over feather development because longer tarsi enable them to better position themselves to outcompete siblings for food delivered by provisioning parents (Mainwaring et al. 2010). Whilst the reduction of investment in wing length may ultimately reduce their manoeuvrability once fledged, the priority at the time of investment is to access resources. Thus, individuals invest in their current condition to increase their chances of surviving to fledging age. In marsh tits (*Poecile palustris*), however, smaller nestlings were found to invest more in wing length, and as such were able to fledge simultaneously with their siblings (Nilsson & Gårdmark 2001); parents preferentially fed fledged chicks over those that remained in the nest, so when the largest nestlings reached the threshold wing length the rest of the brood needed to be ready to fledge too (Nilsson & Gårdmark 2001; Nilsson & Svensson 1996).

Investing primarily in survival may be the best option when resources are limited. During male removal experiments in collared flycatchers (*Ficedula albicollis*), chick growth slowed with the reduction in parental care, but when the resources available to the nestlings increased after the treatment was completed, mass gain was much faster in treated chicks than in controls (Hegyi & Török 2007). Body mass is essential for post-fledging survival, because individuals of this species migrate long distances (Hegyi & Török 2007). However, the same compensation did not occur for skeletal structures and this failure may lead to being outcompeted for reproductive opportunities later in life (Metcalfe & Monaghan 2003).

Another long-term effect caused by early life disturbances in birds is the impact on adult song output (Schmidt et al. 2014; Spencer et al. 2004). The 'developmental stress hypothesis' proposes that because the development of the neural pathways required to learn and produce song are formed during the time when young birds are most susceptible to stress, adult song quality is negatively affected by exposure to stress in early life as there is no way of compensating for this in later life (Buchanan et al. 2003; Nowicki et al. 1998). Early life stress has been shown to reduce song characteristics such as repertoire size in European starlings (*Sturnus vulgaris*; Spencer et al. 2004) and song complexity in zebra finches (*Taeniopygia guttata*; Zann & Cash 2008) and song sparrows (*Melospiza melodia*; Schmidt et al. 2014). It is therefore likely that different growth strategies in nestlings are associated with variation in adult song characteristics, but this has never been tested.

Most experimental studies of nestling growth rates have focused on the impact of hatching asynchrony and body size (e.g. Mainwaring et al. 2010; Stier et al. 2015) or aspects of nutritional stress, including food availability (e.g. Lepczyk & Karasov 2000), sibling competition (e.g. Nilsson & Gårdmark 2001), parental care (e.g. Hegyi & Török 2007) or dietary quality (e.g. Birkhead et al. 1999). However, few studies have investigated these effects in combination, in particular the extent to which nutritional stress may impact on catch-up growth. Furthermore, the consequences of variation in nestling growth rates for song quality in adulthood requires further research, especially in wild bird populations. In this study, I

manipulated the dietary quality of nestlings in order to investigate the relationship between nutritional stress, body size and growth rates in the dipper (*Cinclus cinclus*). It was predicted that smaller birds would grow at a faster rate, but that this relationship would be mediated by dietary quality. I then tested whether nestling growth rates predict adult song quality, focussing on repertoire size and song rate. These two song characteristics have been shown to correlate with early life conditions (Chapter 5); they are thought to be important in mate attraction (Chapter 3) and predict reproductive success (Chapter 6). It was predicted that, due to developmental trade-offs, individuals with higher growth rates would have smaller repertoires and sing at lower rates.

## 7.3 Methods

### *7.3.1 Study population*

Data were collected from an individually marked population of 40-50 pairs of dippers in the River Lune catchment near Sedbergh, Cumbria, UK. For more details of the study population and field methods, see Chapter 2.

### *7.3.2 Experimental procedure*

The dietary manipulation experiment was conducted between March and June 2015 using 16 nests, containing 57 nestlings (13 experimental broods with 48 chicks, 3 control broods with 9 chicks). All trials took place in the morning, when offspring provisioning rates are known to be at their highest (personal observations). All nests were the first to reach the nestling stage for that pair. For each experimental nest, all chicks were removed on days 5, 7, 9, 11 and 13, measured and then hand-fed an experimental diet before being returned (day 0 = the day of

hatching). Chicks from control nests were removed only to be measured, with no diet being administered. This period was chosen partly to minimise disturbance and the risk of premature fledging; females rarely brood their chicks after day 5 (personal observations) when they are less susceptible to cold, and after day 13 the likelihood of chicks leaving the nest when disturbed increases substantially. Moreover, this period also corresponds with the linear phase of nestling growth (Ormerod et al. 1986) and so is a critical period of development. To enable individual identification before ringing on day 9, all nestlings were marked by clipping *c*.1 mm of their claws on a combination of their toes which was unique within the brood. The following biometrics were measured for each chick in every session: (1) 'body mass' taken using electronic scales (±0.1 g); (2) 'tarsus' length (± 0.5 mm) tarsus length was measured using dial callipers using the standard method as described by Svensson (1992); and (3) 'wing' length (± 1 mm) taken from the carpal joint to the end of the longest primary on a closed wing using a capped wing rule.

Within each nest, half of the brood were given a high protein diet and the other half were fed a high fat diet. The former was considered a high-quality diet and the latter a low-quality diet because it would reduce the nutrient intake of the nestlings whilst maintaining energy demand. Diet types were allocated to individuals at random; for broods with an odd number of nestlings, the larger half was given the protein diet at the first nest and the fat diet at the second nest, alternating thereafter. Nestlings were fed their experimental diets until satiation; this was clear from their behaviour as they stopped begging for food, refused to swallow food items offered to them and, in some cases, exhibited a state of torpidity. Forceps were used to place food items at the back of the nestling's throat to mimic the feeding action of an adult, and their natural swallowing reflex enabled them to ingest the food provided. Nestlings on the protein diet were given pieces of raw lean beef mince. On average, individuals consumed 1.9 g, 2.9 g, 3.2 g, 3.6 g, and 4.7 g on days 5, 7, 9, 11 and 13 respectively. The 'fat' diet consisted of small pieces of shredded beef suet (Atora Shredded Suet), and nestlings consumed an average of 0.9 g, 1.4 g, 1.8 g, 2.7 g and 2.9 g on days 5, 7, 9, 11 and 13 respectively.

Nestlings were kept warm during the experiment by use of a hot water bottle and a specially designed woollen carrier. Once satiated, all nestlings were returned to the nest which was then observed for up to 30 minutes from a suitable hiding place at least 10 m away. If a parent attempted to deliver food but returned from the nest with the food load still in its beak, it was deemed that nestlings had successfully been fed to satiation. This occurred in 98% of 80 observations, and in the remainder the parents successfully fed their chicks. This only occurred on two occasions in separate nests; the data from these nests were still used in the analysis as the nestlings were successfully fed to satiation on the other four treatment days and each chick had consumed above the average for their respective diet types during the session in question. Furthermore, it is unlikely that the parent provided food to all nestlings with a single food load.

### *7.3.3 Growth rates*

For every nestling, a growth rate was calculated for each biometric from a linear regression using measurements from all five days. The rate of growth in body mass is known to be linear during this period (O'Halloran et al. 1992; Ormerod et al. 1986) and preliminary analyses revealed similar patterns for tarsus and wing lengths. Sexual dimorphism only becomes apparent when nestlings are around 12-14 days old and even then cannot be used as a reliable method of distinguishing males and females as there is a large amount of overlap between the sexes (O'Halloran et al. 1992).

### *7.3.4 Provisioning rates*

Once hatched, 1 hour nest watches were conducted at each nest on day 2 to measure the offspring 'provisioning rate' by both parents. For more details on methods, see Chapter 2.

### *7.3.5 Territory quality measures*

Primary feeding locations were identified during nest watches as the riffles within each territory where the adults were seen foraging most frequently. Kick samples and in-situ water

sampling were conducted at these locations during the final week of the nestling period. For more information on methods, see Chapter 2.

### *7.3.6 Song recording and analysis*

Song recordings from experimental birds which had recruited into the 2016 breeding population were collected from January to June. For more details of recording methods and sound analysis, see Chapter 2.

### *7.3.7 Statistical analyses*

Linear mixed models (LMEs) were used to test the relationship between early life conditions and nestling growth rates for each biometric (body mass, tarsus length and wing length; Gaussian errors in each case). These explanatory variables were: diet quality (the experimental treatment); initial size (size on day 5 as a measure of nestling condition before the growth period); brood size (as a measure of sibling competition); provisioning rate (as a measure of food availability and parental investment); and ASPT, altitude, DOC, pH and EC (all as measures of territory quality). To control for brood effects, 'brood identity' was included as a random term. Diet was measured as a factor: 'control', 'protein (high quality)' or 'fat (low quality)'; brood size was measured as a factor from values taken from average brood size for nests that year plus or minus one standard deviation (3.91 ± 0.99), so broods with 4 chicks were classed as 'medium', <4 'small' and 4< 'large'; provisioning rate was the sum of both parents' visits during the 1 hour nest watch. Prior to analysis, all variables were centred and standardised to improve interpretation of main effects (Schielzeth 2010). Collinearity between explanatory variables was checked using pair-wise scatterplots, correlation coefficients and variance inflation factors (Zuur et al. 2010).

For all growth rate models, altitude and pH were removed because they were correlated with ASPT (r = 0.6 and -0.5, respectively) and DOC and EC were dropped because of correlations with provisioning rate (r = 0.6 and 0.5, respectively). ASPT and provisioning rate were favoured because they are a well-established, more inclusive measure of water quality and

a useful index of the nutritional stress experienced by nestlings, respectively. In the wing growth rate model, ASPT was dropped because it was positively correlated with brood size ($r = 0.6$) and the latter was prioritised because sibling competition was considered to be a more important driver of stress in nestlings than territory quality. All other variables were considered because correlations were low ($r < 0.4$) and variation inflation factors small ($< 3$). In order to test whether diet quality impacted the relationship between initial size and growth rate, the interaction between diet quality and initial size was also fitted into the models.

LMEs were constructed in R, version 3.2.2 (R Core Team, 2015). For each biometric, the full model was subjected to the 'dredge' function in the package 'MuMIn' (Barton 2016) to rank all sub-models by Akaike's Information Criterion, with the Hurvich and Tsai correction for small sample size (AICc) (Burnham & Anderson 2002; Hurvich & Tsai 1989). If $\Delta AICc \leq 2$ in two or more of the most parsimonious models, model averaging was performed. Models were validated by plotting the distribution of the residuals, the residuals versus the fitted values and the residuals versus each of the covariates (Zuur et al. 2009).

The impact of growth rates on final size was investigated by classifying the rate of growth in each biometric as either 'fast' or 'slow' (greater or less than the mean, respectively) and then testing for a difference in size on day 13 between the two groups using, t-tests and Mann-Whitney U-tests were carried out for normally and non-normally distributed measurements, respectively. The means were as follows: N = 57, body mass growth rate $\overline{x}$ = 3.84g $day^{-1}$; tarsus growth rate $\overline{x}$ = 1.51 mm $day^{-1}$; and wing growth rate $\overline{x}$ = 4.31mm $day^{-1}$. Finally, Spearman's rank correlations were used to test the relationships between nestling growth rates and the two song characteristics (repertoire size and song rate) because sample sizes were small and the data non-normally distributed.

## 7.4 Results

For all biometrics, the best predictor of growth rate was initial size (Tables 1-3, Figures 1-3). As predicted, lighter individuals and those with smaller tarsi on day five had higher growth rates (Tables 1-2, Figure 1-2). However, counter to predictions, individuals with shorter initial wing lengths had slower growth rates (Table 3, Figure 3). These relationships were unaffected by diet quality, which had no effect on nestling growth rates (Tables 1-3). In fact, no other terms were retained in the best fitting models (Appendix 4, Figures 1-3).

There was no difference in day 13 size between individuals with fast and slow growth rates for body mass ($N = 57$, $t = -0.41$, $p = 0.68$; Figure 4a), or tarsus length ($N = 57$, $t = -1.16$, $p = 0.25$; Figure 4b). By contrast, nestlings with fast growth rates had significantly longer wing lengths than individuals with slow growth rates ($N = 50$, $t = 5.1$, $p < 0.001$; Figure 4c).

There was no relationship between body mass growth rate and adult repertoire size ($N = 6$, $r = -0.54$, $p=0.30$), but there was a significant positive correlation between the rate of growth in body mass and adult song rate ($N = 6$, $r = 0.81$, $p=0.05$; Figure 5). There were no correlations between tarsus growth rate and adult repertoire size ($N = 6$, $r = -0.31$, $p = 0.56$) or song rate ($N = 6$, $r = 0.32$, $p = 0.54$). Finally, the rate of growth in wing length was significantly negatively correlated with adult repertoire size ($N = 6$, $rho = -0.88$, $p=0.02$, figure 6), but there was no relationship between wing growth rate and adult song rate ($N = 6$, $rho = 0.09$, $p = 0.86$).

**Table 1.** The model selection table for the global model of an averaged linear mixed model examining the factors associated with body mass growth rate:

lmer(body mass growth rate ~ brood size + ASPT + initial body mass + provisioning rate + diet + EC + initial body mass * brood size + initial body mass * diet + initial body mass * provisioning rate + ( 1| brood identity )

| # | (Intercept) | ASPT | Brood size | Diet | EC | Provision rate | Initial body mass | Initial body mass * Brood size | Initial body mass * Diet | Initial body mass * Provision rate | df | logLik | AICc | delta | weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 33 | 3.87 | | | | | | -0.16 | | | | 4 | -28.70 | 66.24 | 0.00 | 0.56 |
| 1 | 3.87 | | | | | | | | | | 3 | -30.80 | 68.09 | 1.85 | 0.22 |

**Table 2.** Model selection table for global model:

lmer(tarsus growth rate ~ brood size + ASPT + initial tarsus length + provisioning rate + diet + initial tarsus length * brood size + initial tarsus length * diet + initial tarsus length * provisioning rate + ( 1| brood identity )

| # | (Intercept) | ASPT | Brood size | Initial tarsus length | Diet | Provision rate | Initial tarsus length * Brood size | Initial tarsus length * Diet | Initial tarsus length * Provision rate | df | logLik | AICc | delta | weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 1.52 | | | -0.15 | | | | | | 4 | 30.73 | -52.62 | 0.00 | 0.95 |

**Table 3.** Model selection table for global model:

lmer(wing growth rate ~ brood size + initial wing length + provisioning rate + diet + DOC + initial wing length * brood size + initial wing length * diet + initial wing length * provisioning rate + ( 1| brood identity )

| | (Intercept) | Brood size | Diet | Provision rate | Initial wing length | Initial wing length * Brood size | Initial wing length * Diet | Initial wing length * Provision rate | df | logLik | AICc | delta |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **17** | 4.31 | | | | 0.13 | | | | 4 | -0.73 | 10.44 | 0.00 |
| **1** | 4.30 | | | | | | | | 3 | -2.19 | 10.95 | 0.51 |

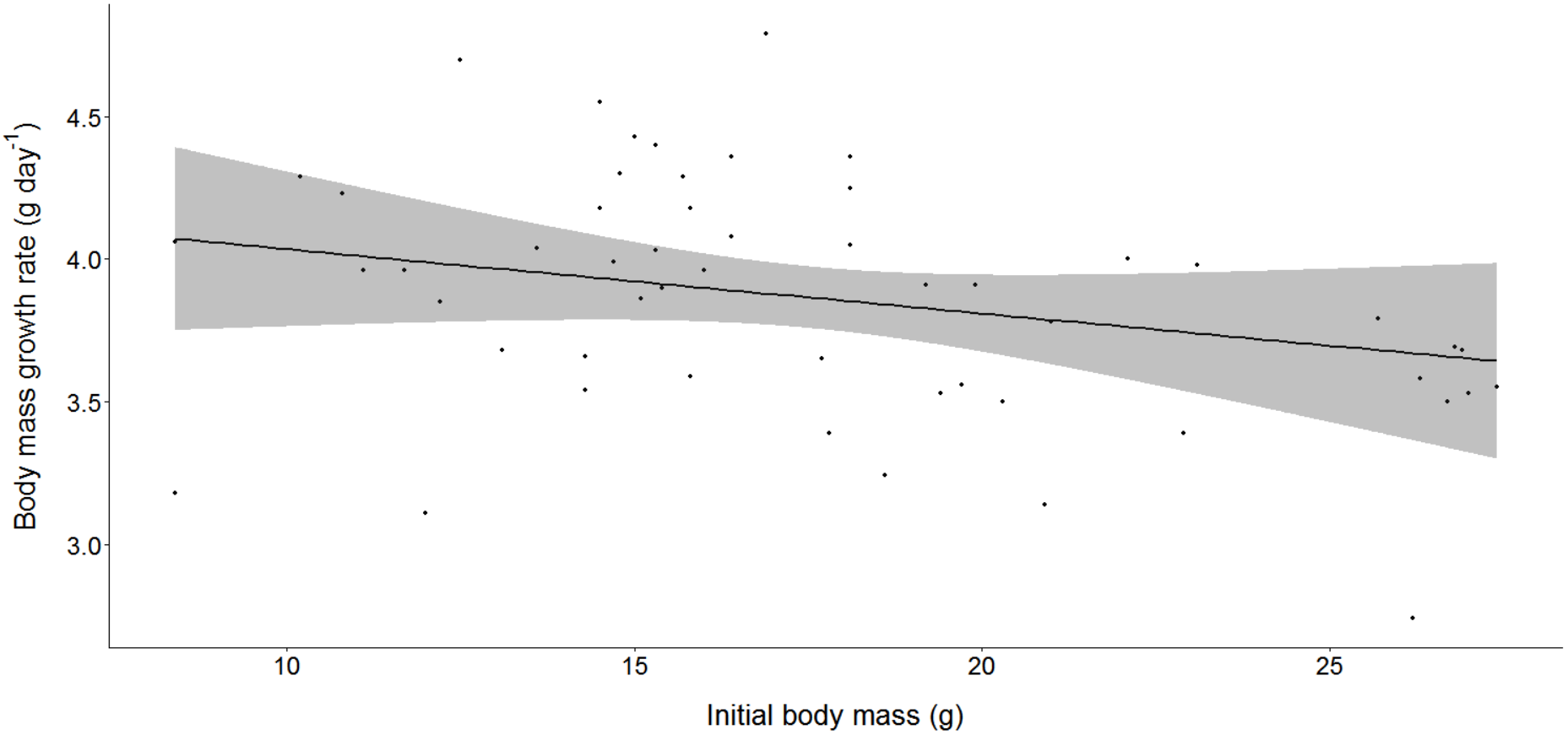


**Figure 1.** The relationship between initial body mass in nestlings and growth rate for body mass, as predicted by the averaged model. The 95% confidence interval is shown in grey, points show the raw data.

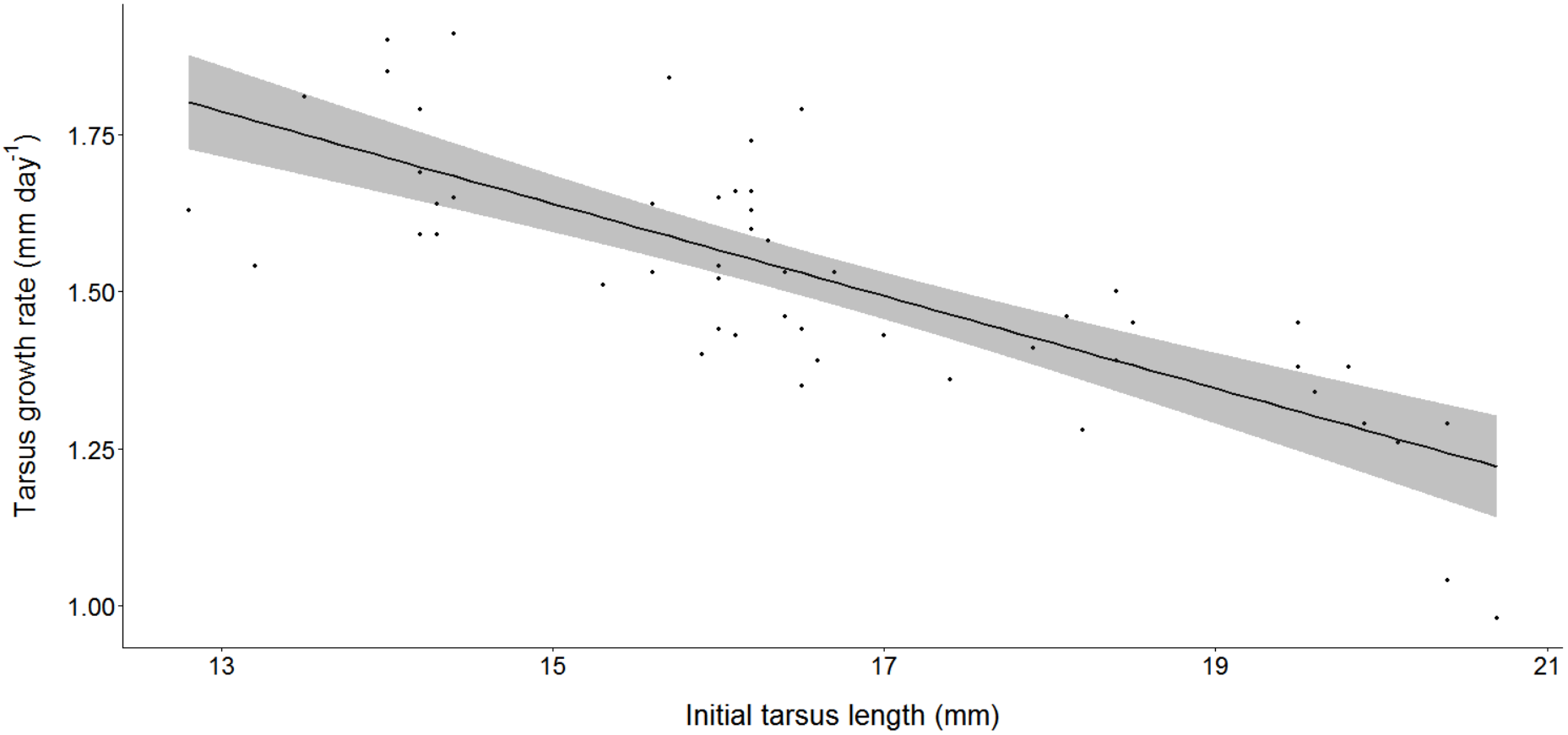


**Figure 2.** The relationship between initial tarsus length in nestlings and the growth rate for tarsus, as predicted by the averaged model. The 95% confidence interval is shown in grey and points show the raw data.

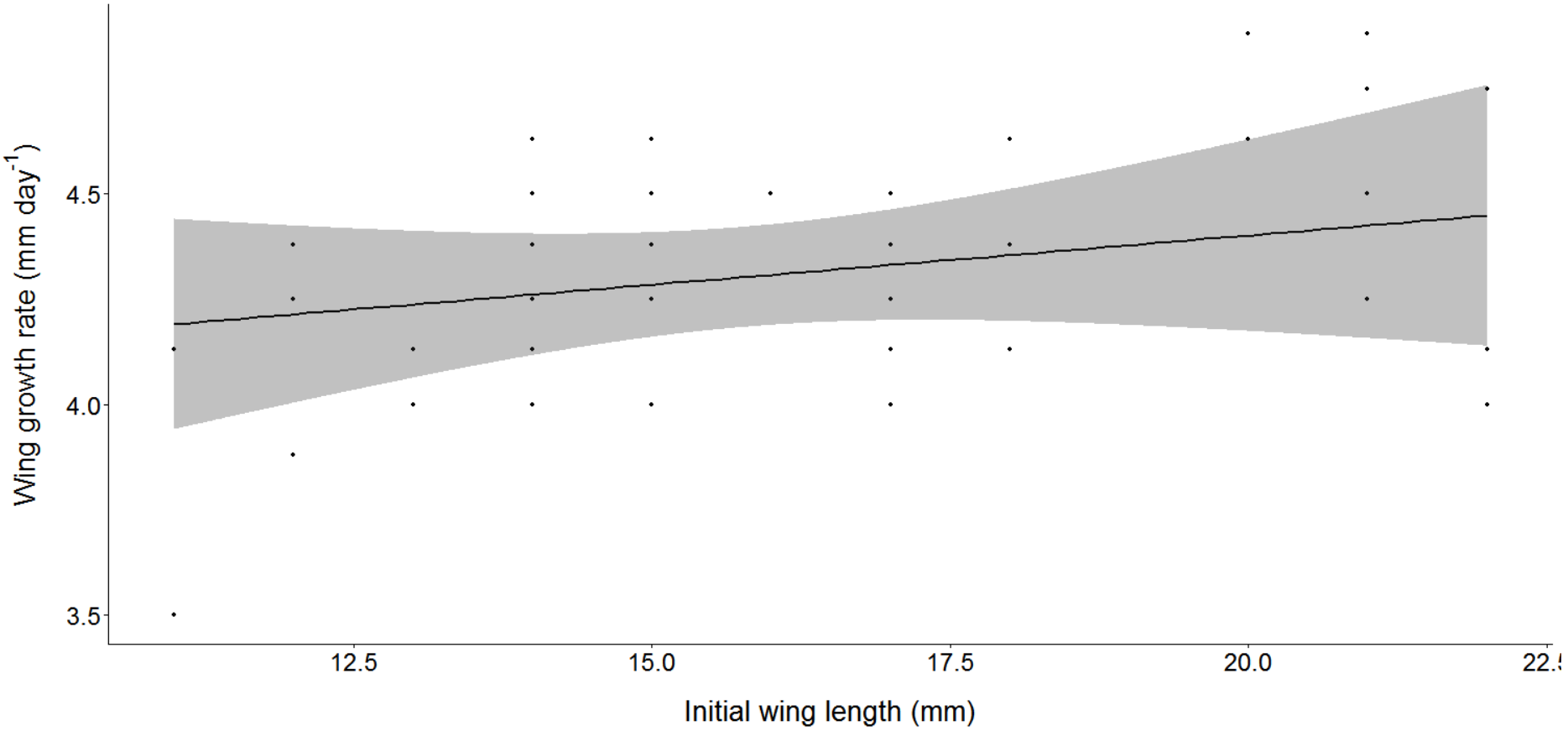


**Figure 3.** The relationship between initial wing length in nestlings and the growth rate for wing, as predicted by the averaged model. The 95% confidence interval is shown in grey and points show the raw data.

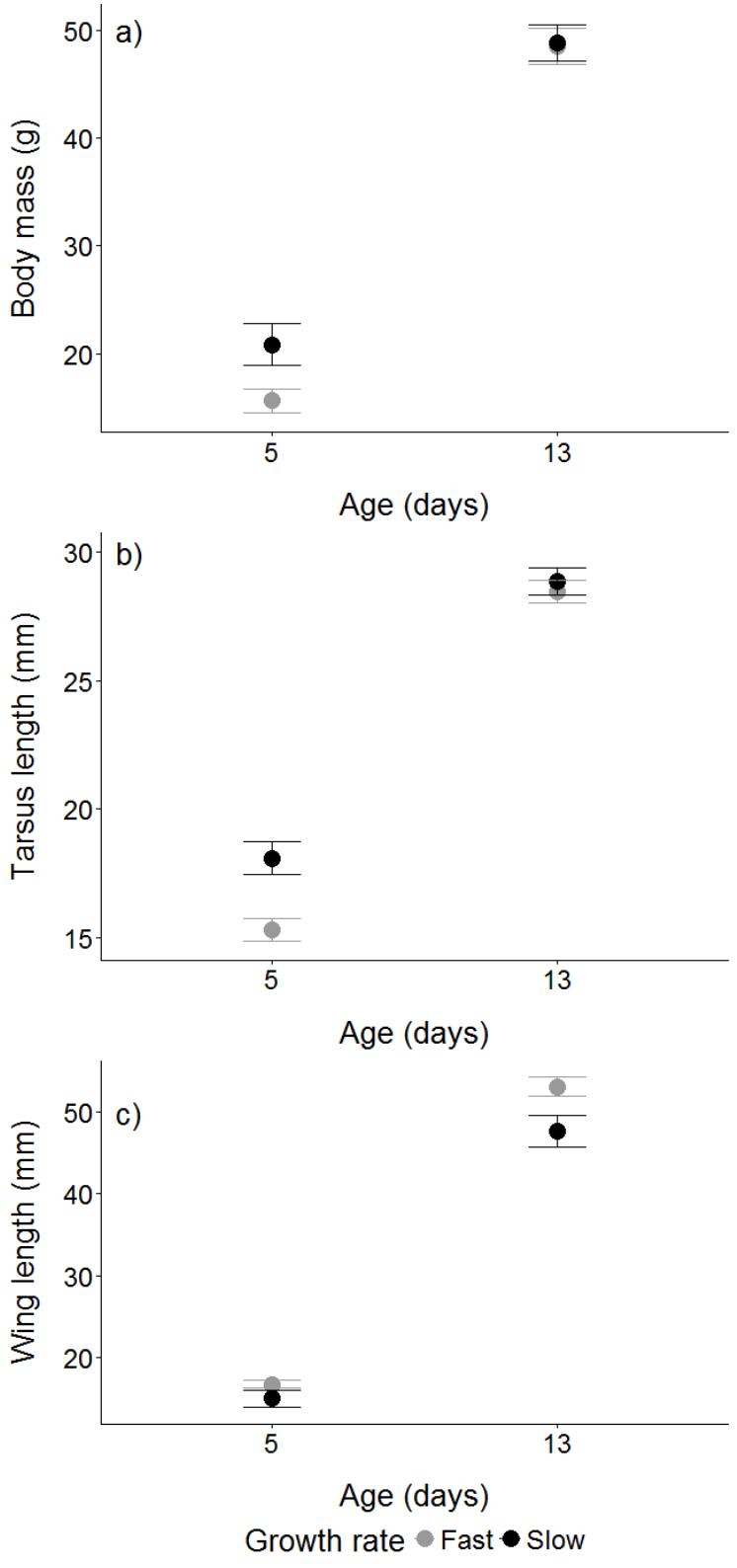


**Figure 4.** Biometric measurements on day 5 and day 13; showing individuals with fast growth rates (grey) and slow growth rates (black) for: **a)** body mass, **b)** tarsus length and **c)** wing length. The points indicate the means and the error bars show one standard error.

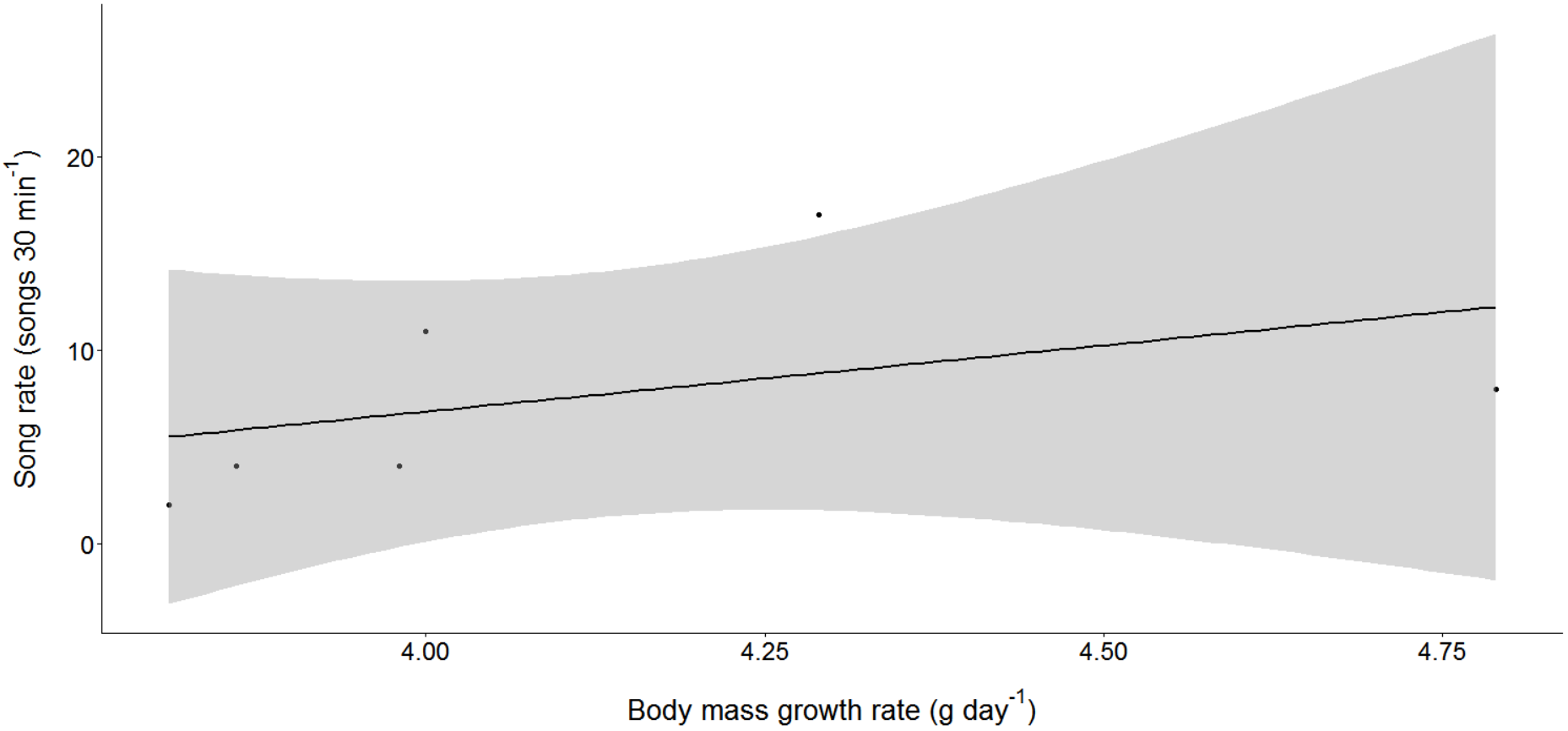


**Figure 5.** The relationship between nestling growth rate in body mass and adult song rate, with one standard error (grey).

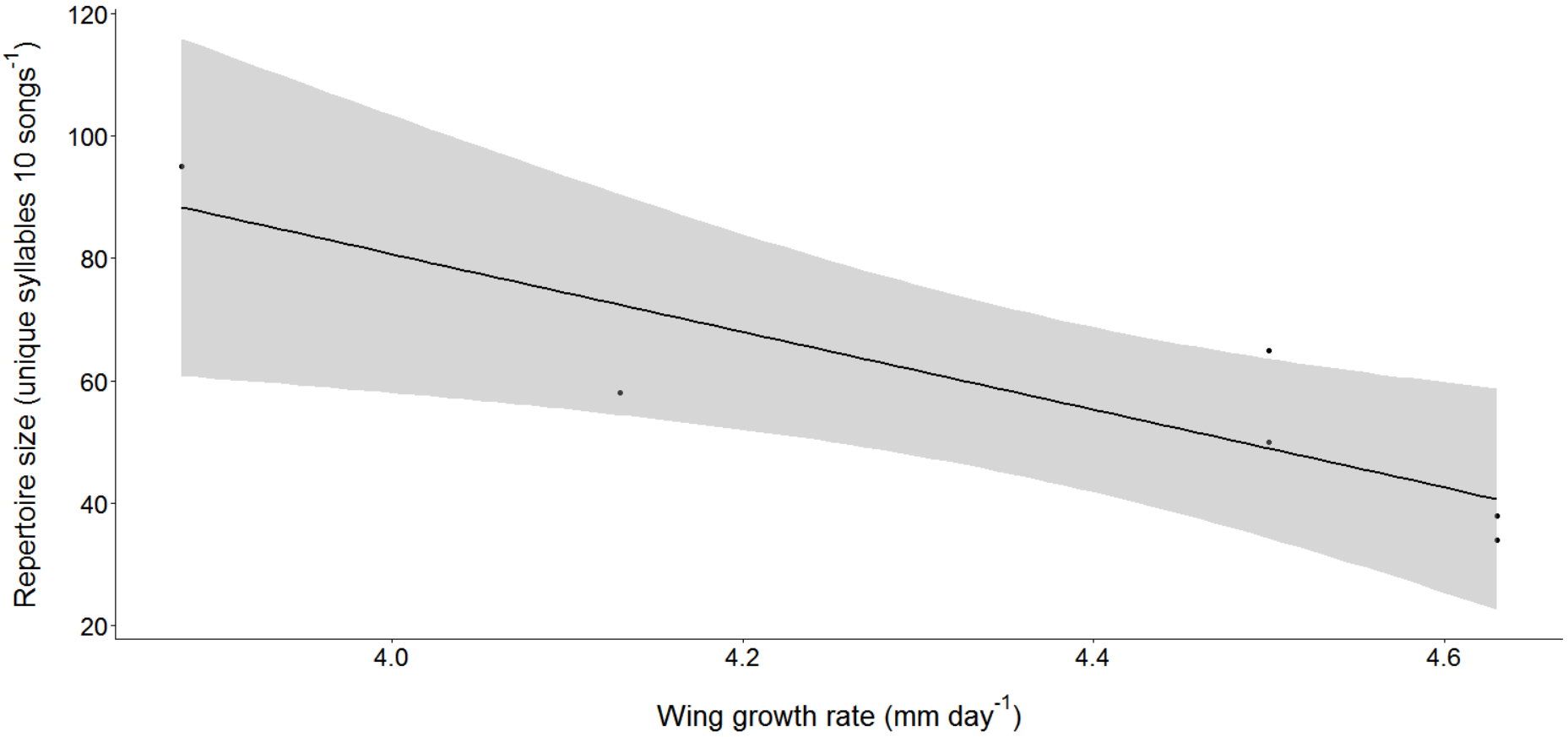


**Figure 6.** The relationship between nestling growth rate in wing length and adult repertoire size, with one standard error (grey).

## 7.5 Discussion

The experimental dietary manipulation did not have any effect on growth rates in nestlings, with the growth rate in all three biometrics best predicted by an individual's size at the start of the growth period. This may provide evidence that the first few days of an individual's life, and perhaps even maternal investment or environmental conditions prior to hatching, have the greatest impact on growth trajectory. The starting size of an individual can be influenced by many things including: maternal investment in eggs (Cunningham & Russell 2000; Krist 2011; Williams 1994), hatching asynchrony (Magrath 1990), food availability (Criscuolo et al. 2008) and sibling competition (Nilsson & Svensson 1996; Verhulst et al. 2006). Maternal investment in eggs has been observed not only to affect an individual's size, but also growth and survival probability (Christians 2002; Reed et al. 2009). In this study, initial size predicted an individual's growth trajectory and therefore data relating egg size to nestling size in the first week of life would help differentiate between the influence of maternal investment in the egg and parental provisioning of hatchings on the growth rate of nestlings (Christians 2002). Hatching order is highly likely to determine the dominance hierarchy and size variation within a brood (Magrath 1990; Mainwaring et al. 2010; Nilsson & Gårdmark 2001), but these data were not available here.

Initial size was negatively correlated with growth rates for body mass and tarsus length; these relationships support the prediction that small individuals invest in compensatory growth. However, there was a positive correlation between initial wing length and the rate of wing growth, and size-based resource allocation therefore varies between different parts of the body. It is possible that dippers exhibit a similar pattern to that observed in blue tits, in which late-hatched nestlings that were smaller and lighter relative to their siblings preferentially invested in tarsi at a cost to feather growth (Mainwaring et al. 2010). This was thought to enable them to better position themselves within the nest cup and therefore increase their competitive ability

and chances of obtaining food. This trade-off seems logical because if nestlings are unable to compete with their siblings for food, they are more likely to starve before fledging or have reduced post-fledging survival (O'Connor 1979; Parsons 1975). Individual dippers that invested in faster body mass and tarsus growth rates were able to achieve similar sizes by day 13 as those in which growth rates were slower, suggesting that compensatory growth was successful. This investment may also have long-term benefits; a significant positive correlation was found between the rate of growth in body mass and song rate in adulthood, which is commonly associated with the current condition of an individual (Grunst & Grunst 2014). These results therefore support previous findings that body condition during development impacts on adult condition (Criscuolo et al. 2008; Krause et al. 2009) and that early life investment in body size is reflected in song output (Soma et al. 2006).

Smaller individuals had slower wing growth rates and, by day 13, still had shorter wings than larger individuals with faster growth. First-year dippers have significantly shorter wing lengths than adult dippers and this is thought to be due to a lack of foraging expertise or undernourishment in the nest, but shorter wing lengths are also thought to provide them with greater manoeuvrability which is beneficial to predator evasion (Tyler & Ormerod 1994). In Chapter 6, wing length was shown to be a significant determinant of repertoire size in adult males; individuals with shorter wing lengths had larger repertoires. The negative relationship shown here between wing growth rate and repertoire size may provide additional support to the idea that differential investment occurs during development, with individuals investing either in body size or the necessary brain structures required to develop complex songs, but rarely have the resources required to invest in both. Larger individuals invest in longer wings, which may be a necessity for their body size (Hamilton 1961), but this may mean that fewer resources can then be invested in neural song regions. Equally, birds may invest in body size over singing ability, as both are determinants of reproductive success and the ability to obtain a mate (Grant & Grant 2000; Johnson & Johnston 1989). These apparent trade-offs did not vary with diet quality, but it is possible the amount of food provided was not enough to make a difference.

The low sample size of control nests made it difficult to detect any differences between control and experimental broods and the small number of individuals which recruited into the 2016 breeding population (control: N = 1; protein: N = 4; fat: N = 1) meant that it was not possible to determine any long-term effects of diet quality in early life on song quality. A follow up study using a more intensive feeding regime, with more visits per day and more consecutive days feeding may increase any effect on growth rates and reduce the potential impact of compensatory parental feeding which may occur for nestlings given the low-quality diet. Diet type was chosen with the idea that fat fed chicks would lack the sufficient nutrients to invest in growth, however it is possible that parental provisioning compensated for this and the chicks suffered no detrimental effects. Food restriction may have had a greater influence (Lepczyk & Karasov 2000), but this is difficult to conduct within the field and it was predicted that once the chicks were returned to the nest after starving half a brood, these individuals would increase begging and once again parental provisioning would compensate for the food restriction in these individuals.

## 7.6 Conclusion

This is one of the first field studies to investigate the relationship between nestling growth rates and adult song characteristics in wild birds. Despite small sample sizes, the results show a significant positive correlation between the rate of growth in body mass and song rate, and a significant negative correlation between wing growth rate and repertoire size. These relationships provide clear evidence to support the hypothesis that growth rates in early life have far-reaching consequences, not just for survival but for other components of fitness. In this study, nestling growth rates were found not to correlate with various early life conditions but

instead were largely driven by initial size. Therefore, future work should focus on what determines an individual's size during the first few days of life, and the extent to which maternal investment and nutritional stress at that time may influence song characteristics in adulthood. Further research is also needed to investigate the relative timing of growth of different structures in order to determine whether a trade-off between body size and neurological song regions is occurring.

# Chapter 8

# General discussion

## 8.1 Introduction

Very little was previously known about the song of dippers beyond the observation that both sexes produce complex song, males singing more frequently throughout the breeding season and females not singing whilst on the nest (Cramp & Simmons 1988; Tyler & Ormerod 1994). This project provides the first detailed description of the structure and function of dipper song, and is one of the first studies of a wild bird to investigate the early life conditions associated with structural variation in song together with the fitness consequences of that variation.

The 'developmental stress hypothesis' predicts that the quality and quantity of song produced by an individual are an accurate representation of its developmental history (Buchanan et al. 2003; Nowicki et al. 1998). Development of the brain nuclei associated with song learning and production occurs at a time when young birds are exposed to a high number of potential stressors, including malnutrition, parasites or adverse weather conditions (Buchanan et al. 2003; Nowicki et al. 1998). These stressors can result in a range of detrimental effects to an individual's development which cannot be compensated for in later life (Buchanan et al. 2003; Nowicki, W A Searcy, et al. 2002), and only high quality individuals can afford to invest in both song development and growth or survival (Nowicki, W A Searcy, et al. 2002). Song characteristics may therefore provide the listener with information about an individual's quality and the level of stress experienced during early life. There are many examples of the effect

developmental stress has on adult song characteristics in captive birds, but studies on wild bird populations are rare.

The first aims of this study were to observe and record song and the singing behaviour of male and female dippers, to compare song between the sexes and to better understand the function of song in this species. Once key song characteristics had been identified, the development of these parameters was examined by testing for correlations with a range of early life conditions. Following this, the importance of song in adult male dippers was assessed, testing for associations between song complexity or song rate and various measures of body condition and reproductive success. Finally, further work was conducted to investigate the effect of nutrition and growth in early life on song development.

## 8.2 Overview of results

In Chapter 3 I looked at the structural differences between male and female song at different breeding stages and the seasonal variation in singing. Female song was similar to that of male song but showed higher versatility, a measure of complexity, and a lower number of syllables per second. Female song was used most frequently during aggressive interactions with individuals from outside of their territory. In males, song characteristics were found to be dependent upon the reproductive stage and paired status of the individual recorded. Unpaired individuals recorded early in the season had larger repertoires and higher song rates, thus providing evidence that male song is primarily used for mate attraction. Males sang throughout the breeding season, whereas females were rarely observed to sing after laying, probably due to investment in breeding activities such as incubation and brooding. Singing in both sexes peaked at the start of the season when disputes for breeding territories were ongoing. Overall, it can be

concluded that both sexes use song to defend resources, whether it be a mate or territory. There was little evidence that female song was used for mate attraction, but the similarity in acoustic structure with male song raises questions about whether female song is a sexually selected trait. Song may also function to coordinate breeding activities to maximise reproductive success, but again further work is needed to investigate this.

Experimental playback trials were conducted to further compare the function of male and female song, and the results were discussed in Chapter 4. There was no difference in the responses of males or females to simulated intruders of either sex whether trials were conducted before or after breeding had started. This leads me to believe that a pair will defend their resources from an intruder regardless of the sex, and the fact that timing does not affect this means that it is more likely to be resource based than particularly for mate defence. Though sample sizes were small, a trend for intrasexual aggression could be seen in both sexes, so it is possible that pairs do not defend resources cooperatively, but individuals act aggressively to increase their own reproductive success, decreasing the incidences of extra-pair copulations. Males take a dominant role in defence as they were observed to respond faster and in more trials than females; this may be because of the constraints imposed by the higher investment in reproduction by females.

In Chapter 5 the quality and quantity of song produced by individuals that had recruited into the breeding population were investigated in order to test whether these song characteristics were predicted by early life conditions. There was a small but significant effect of brood size on repertoire size, with nestlings from smaller broods producing songs with greater complexity. This may be because sibling competition reduces the extent to which individuals can invest in song development. Provisioning rate predicted song rate, with individuals that were fed more frequently by their parents singing at a higher rate as adults; this effect was greater for chicks that started small, or those reared within smaller broods, presumably because an increase in food availability through parental care has a proportionally smaller effect for larger birds or birds in larger broods. Together, these results support the developmental stress hypothesis,

providing further evidence for the long-term detrimental effects of poor early life conditions on adult phenotypes. Male song can therefore provide females with information about the male's developmental past and current condition. Few other studies have shown a link between early life conditions and song characteristics in a wild bird population, but experimental manipulation of these conditions is now needed to untangle the causal mechanisms involved.

In Chapter 6, I investigated the consequences of individual variation in song characteristics for reproductive success. Males with larger repertoires were found to have greater reproductive success, with higher levels of fledging success and more chicks fledged per brood. Larger repertoires may enable males to obtain and defend better quality territories or attract higher quality mates, thereby breeding more successfully. Song rate was highest in unpaired individuals recorded early in the season, but these individuals had lower fledging success and produced lighter chicks. This could reflect their inexperience or be due to the later onset of breeding (because they were unpaired at the start of the year) if this results in a temporal mismatch with the peak in food availability. These results support the idea that song complexity indicates quality in a male and influences his reproductive success. The rate of singing, however, may be more indicative of current status or condition rather than quality, and further analysis is needed of songs recorded from the same male but in different contexts in order to better understand the negative correlation between song rate and reproductive success.

The dietary manipulation experiment described in Chapter 7 aimed to test whether the quality of a nestling's diet impacted on adult song characteristics via its effect on growth rates in early life. However, diet quality had no effect on nestling growth rates, with the initial size of an individual being the major determinant of its growth trajectory. Too few experimental individuals recruited into the breeding population in order to investigate the link between diet and song directly, but the rate of growth in the body mass of nestlings correlated positively with adult song rate. This could be an indication of the importance of investing in future condition, because this too can affect secondary sexual characteristics such as song production. The growth rate of wing length in nestlings was negatively correlated with repertoire size in

adulthood, supporting the findings from Chapter 6 that individuals with smaller wing lengths have larger repertoire sizes. This may be the result of a trade-off in investment, with smaller individuals investing more in song nuclei than feather growth in order to maximise their reproductive success.

## 8.3 Conclusion

The developmental stress hypothesis suggests that song development can be impaired when an individual is exposed to stress during the early stages of life (Buchanan et al. 2003; Nowicki et al. 1998), but evidence from wild populations is lacking. In birds, the nestling and fledgling stages are periods when individuals are most vulnerable to stress (Nowicki et al. 1998). Dippers make an ideal model system for testing this hypothesis in the wild because they defend linear territories year-round and so are easy to locate and monitor, and they feed almost exclusively on freshwater invertebrates and so are highly sensitive to water quality (Sorace et al. 2002). Nestlings raised on territories which vary in food availability are therefore likely to experience very different levels of nutritional stress.

I found that an individual's early life conditions predicted the quality and quantity of song produced as an adult, supporting the developmental stress hypothesis (Buchanan et al. 2003). When investigating nestling growth trajectories, I found that the allocation of resources differed between chicks of different sizes. Nestlings that started small invested in high rates of growth for body mass and tarsus length, presumably to catch up with their larger siblings, whereas larger nestlings invested more in feather growth (i.e. wing length). These findings are consistent with previous research showing that resource allocation is dependent upon the size of the individual and that investments either improve current condition or long-term fitness (Stier

et al. 2015). Birds with smaller wings were also found to have larger repertoires, which differs from the majority of work on repertoire size or song complexity which are generally thought to be honest signals of male quality (Catchpole & Slater 1995). It is possible that nestling's trade-off their investment, with smaller birds compensating for their size by investing more heavily in song development. This suggests that important life history trajectories may be determined in the first few days of their life, or even pre-hatching by maternal investment (Cunningham & Russell 2000; Reed et al. 2009).

The finding that song is affected by the developmental conditions an individual is reared in is particularly important because of the consequences of song structure and singing behaviour for reproductive success. In dippers, males with larger repertoires had higher breeding success, supporting previous work on the consequences of elaborate song production and the benefits of investment in exaggerated characteristics (Catchpole 1987; Hiebert et al. 1989; Lambrechts & Dhondt 1986; Potvin et al. 2013). The situation is less clear in females. The fact that dippers are monogamous, defend territories year-round and will stay together year after year aligns with previous findings about the incidence and likely function of female song (Illes 2015; Langmore 1998). Song is likely to advertise ownership of a territory or a mate, and joint territorial defence may allow each member of a pair to defend their own reproductive resources but also benefit from extra-pair copulation if the opportunity arises (Hall 2004; Langmore 1998; Morton 1996). However, the small sample size for recordings of female song precludes further analyses of the implications of song structure for breeding success or the influence of early life conditions on song development.

Songs recorded from males actively seeking mates contained a larger number of unique syllables and were given at a higher rate. This suggests that these traits are important for mate attraction and that females may obtain information about a male's developmental past and current condition from his song (Buchanan et al. 2003; Nowicki et al. 2000). However, the finding that song characteristics vary with context also has major implications for future work on the structure and function of song; studies which compare the songs of different males need

to account for the breeding status of the individuals recorded and, ideally, record the songs of the same males at different stages of their lives.

While studies of wild bird populations are essential for better understanding the impact of developmental stress on song development and therefore fitness, they do face different challenges to laboratory work. The dietary manipulation experiment in Chapter 7 was originally conducted in order to test the effect of nutritional stress in early life on song complexity and output. However, in 2016, the level of recruitment into the breeding population was lower than in any other year of the dipper project. This is thought to be mainly the result of Storm Desmond in winter 2015, one of the century's most severe hydrological events (Marsh et al. 2016). The high winter water levels will have made it extremely difficult for dippers to feed (Taylor & O'Halloran 2001) and will have greatly reduced the availability of freshwater invertebrates (Chiu et al. 2013). Young and inexperienced foragers are likely to have suffered the most, and only six experimental birds (10%) were found breeding within the field site the following year. Therefore, the ability to detect the long-term effects of nutritional stress on the song characteristics of these individuals was severely reduced. Future work may include a repeat of this experiment, but with more extensive searches for breeding birds beyond the study site; previous work has shown that natal dispersal distances in male dippers is around 3 km, with females moving more than 5 km on average away from their natal sites (Tyler et al. 1990).

Further areas to expand upon would include more in-depth work on repertoire sizes. Individual dippers are capable of producing an astonishing number of different syllables – 146 being the maximum recorded for a male in this study. This makes it extremely challenging to quantify 'true' repertoire size. The issues I had with finding an individual's maximum repertoire size were due to lack of data. Plots of the cumulative number of unique syllables recorded against the total number of syllables analysed did not show a plateau, meaning that for some individuals the sample of recordings was far from complete. The decision was made to quantify repertoire size using 10 songs per individual, and these analyses established that the context in which an individual's songs are recorded can influence the characteristics of the song.

Therefore, further work on dippers should include songs from multiple contexts per individual, which is likely to mean recording birds throughout the year. In my project, song recording effort was conducted in such a way as to maximise the sample size of individuals; once a sufficient number of recordings were obtained for a given bird, that individual was rarely recorded again. In the future, it would be ideal to record the songs of males when they are unpaired but also later once they have found a partner. It would also be particularly interesting to investigate whether repertoire size changes with age; it is currently unknown whether dippers are open- or close-ended learners, but the large number of syllables that they can produce suggests the former, and this would make them an excellent model system for future studies of song learning.

The increased measures of reproductive success shown for males utilising large repertoires shows that vocal communication within dippers is important and signals portraying quality are being received. These traits are hard to fake because the necessary brain structures are required to produce a high number of different syllables, and as chapter 5 shows, the conditions during early life can have a detrimental effect on adult phenotype. These findings contribute to signalling theory because males honestly communicate their current state and developmental history with song rate and complexity. High quality males therefore benefit from access high quality females or territories and females can select a mate based on his characteristics.

The range of frequencies used to produce vocalisations in dippers is kept above 1 kHz, which shows the use of spatial release from their noisy aquatic environments, increasing the probability of their signal being received. However, during playback experiments the use of aggressive behaviours, such as flights flights over the speaker, and the frequent dipping and blinking that are associated with the dipper itself gives rise to the idea that multimodal signalling is also at work here. Use of visual signals during aggressive and non-aggressive interactions increases the signal strength which is particularly important in an environment with a high level of background noise. With possibility of rising water levels, or increased exposure

to anthropogenic noise, individuals best able to maximise their signal will succeed. Further work on use of visual signals and variation in territory background noise levels would shed further light on the use of multimodal signalling in dippers.

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# Appendices

## Appendix 1

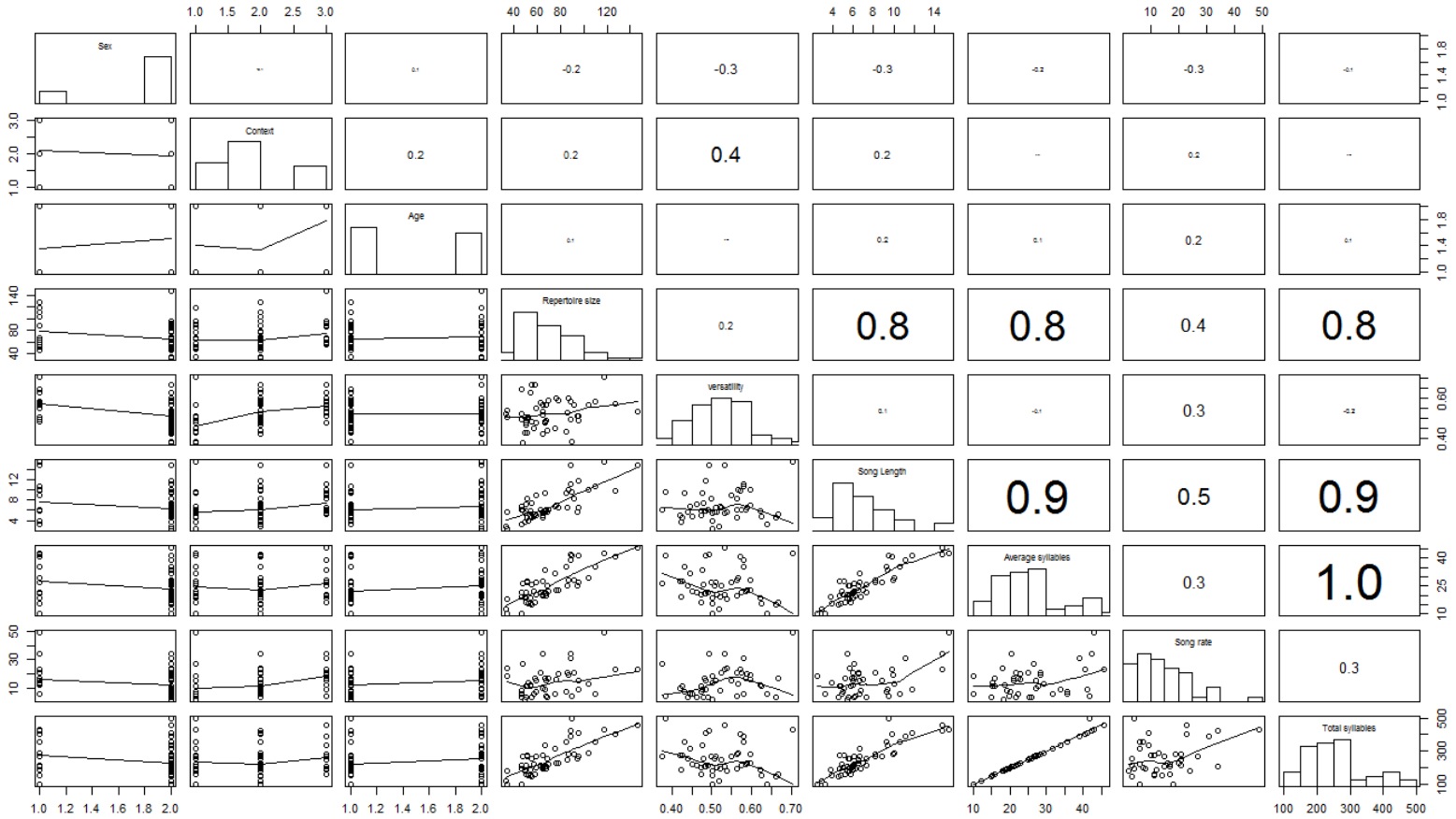


**Figure 1.** Pair plot showing the interactions between all measured song characteristics.

**Appendix 2**

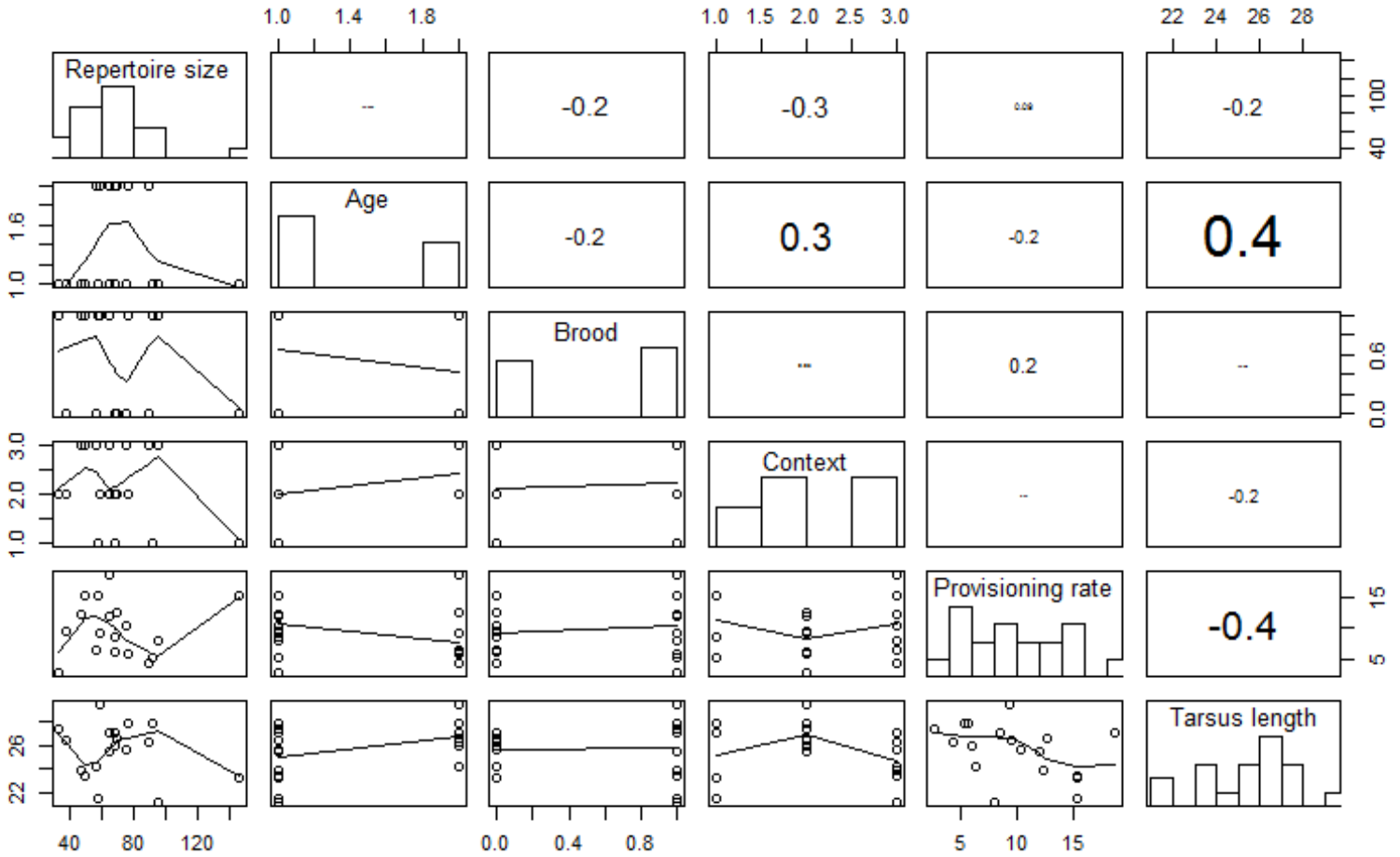


**Figure 1.** Pair plot showing interactions between factors featured in the model determining the effect of early life conditions on repertoire size in adults.

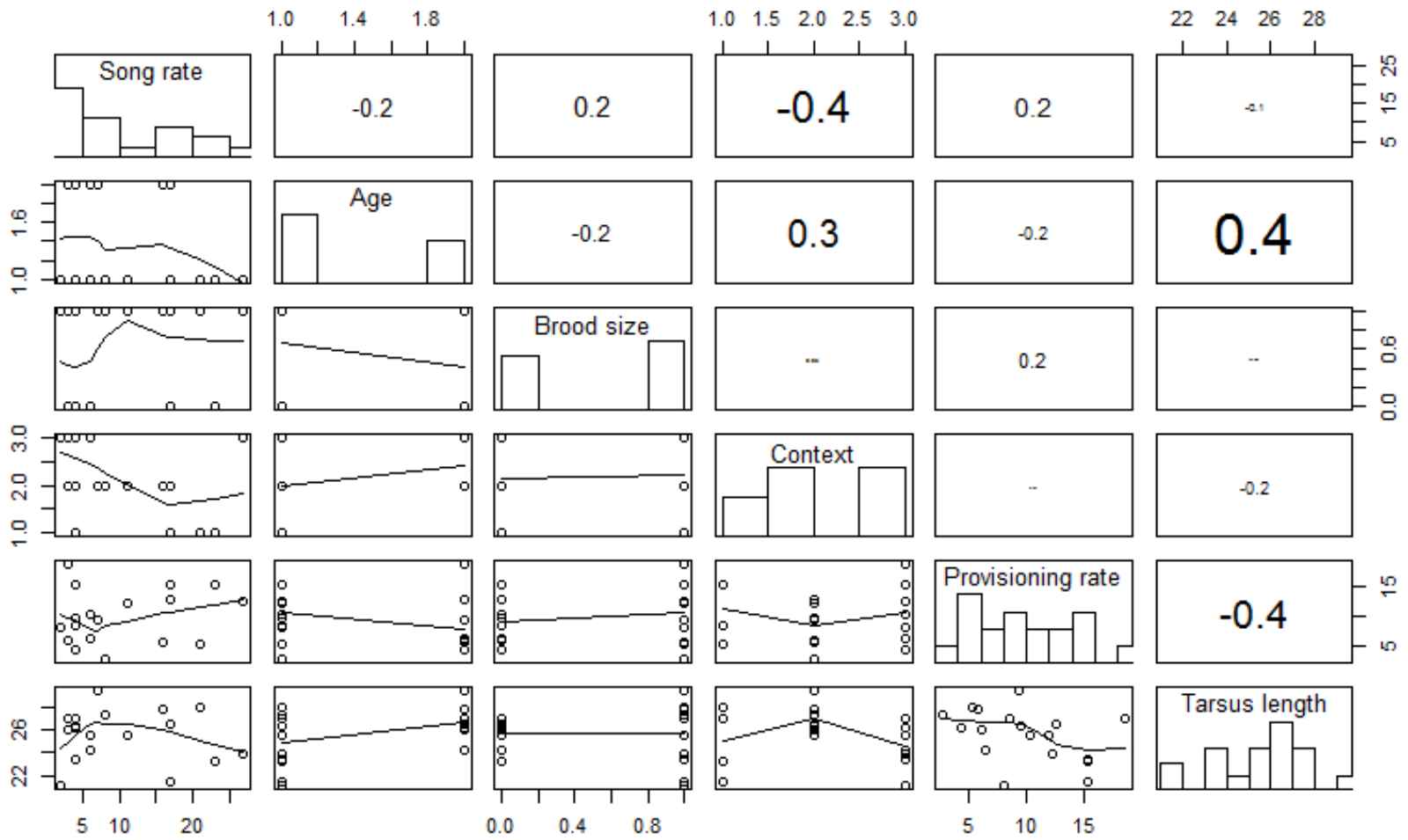


**Figure 2.** Pair plot showing the interactions between factors featured in the model examining the effect of early life conditions on song rate in adults.

**Appendix 3**

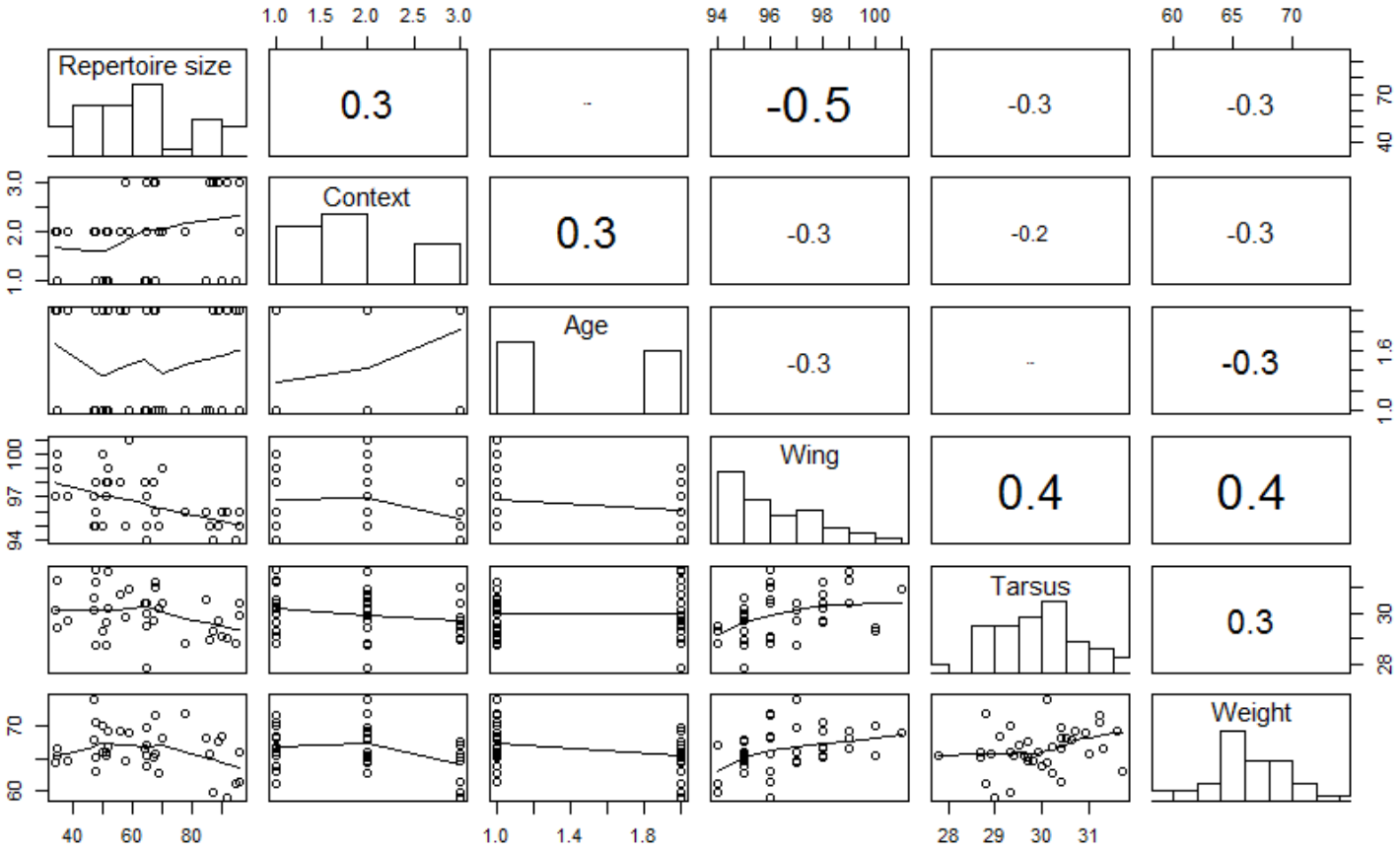


**Figure 1.** Pair plot showing the interactions between the variables used the model which investigated repertoire size.

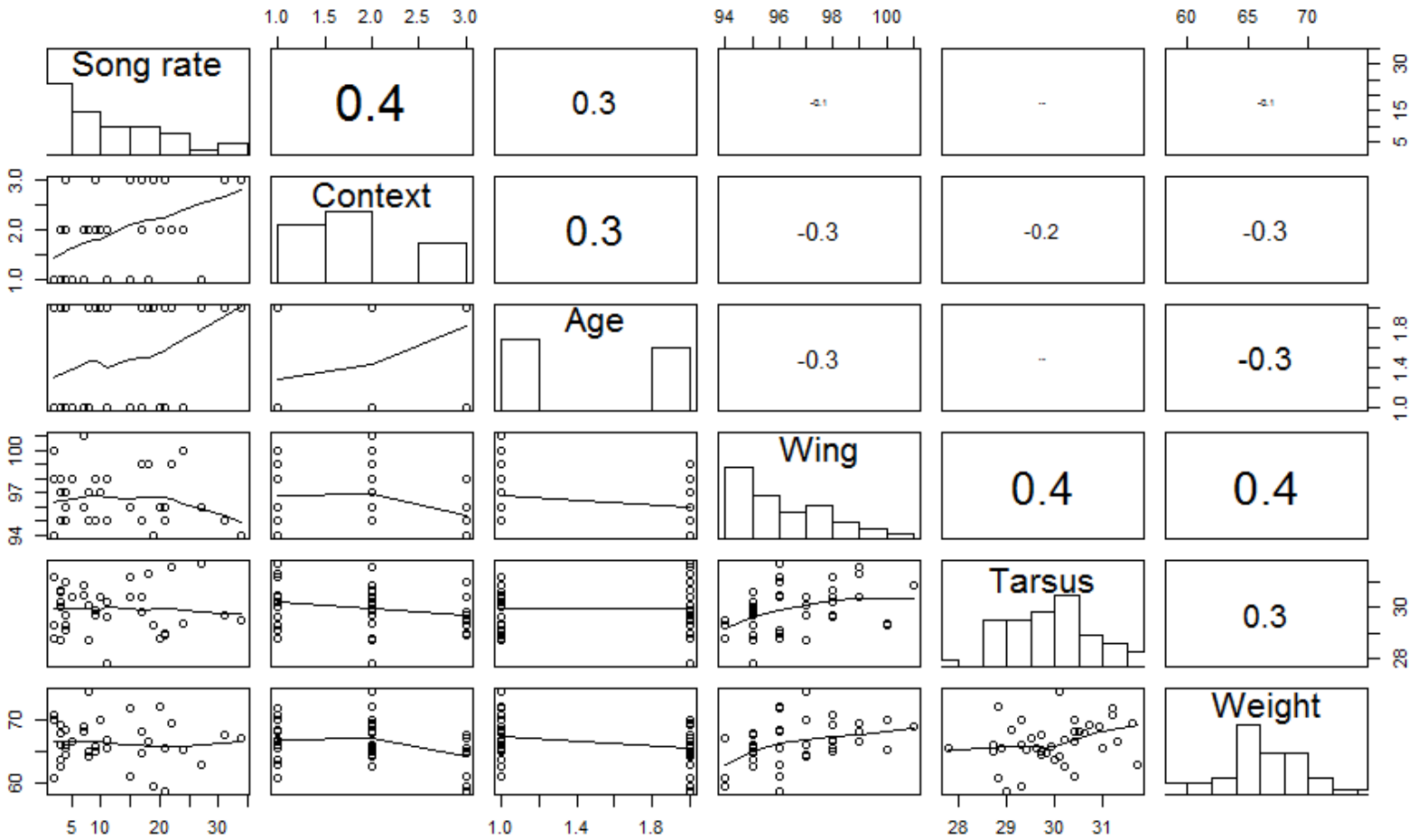


**Figure 2.** Pair plot showing the interactions between the variables involved in the model investigating song rate.

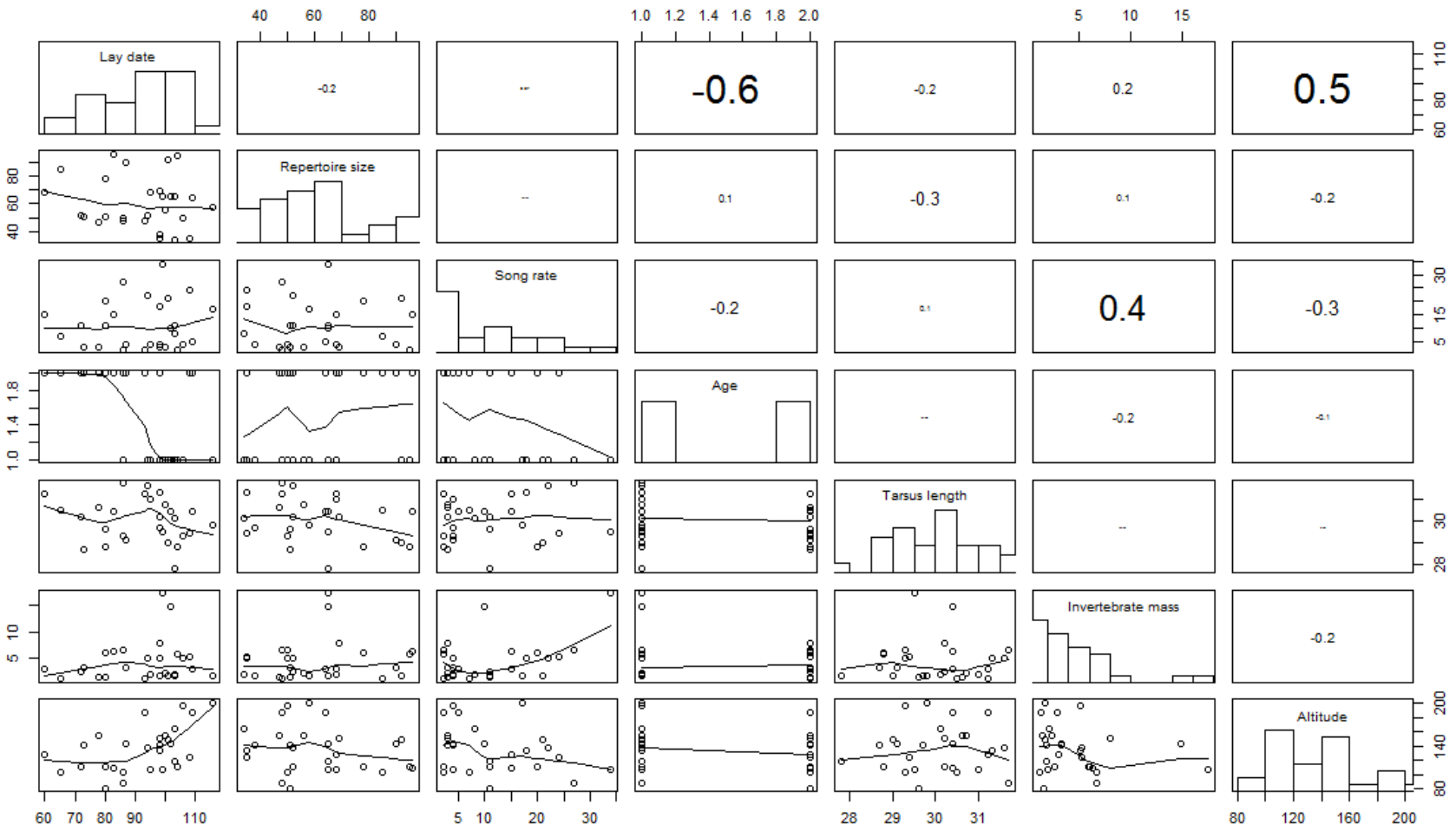


**Figure 3.** Pair plot showing the interactions of all the variables involved in the model investigating lay date.

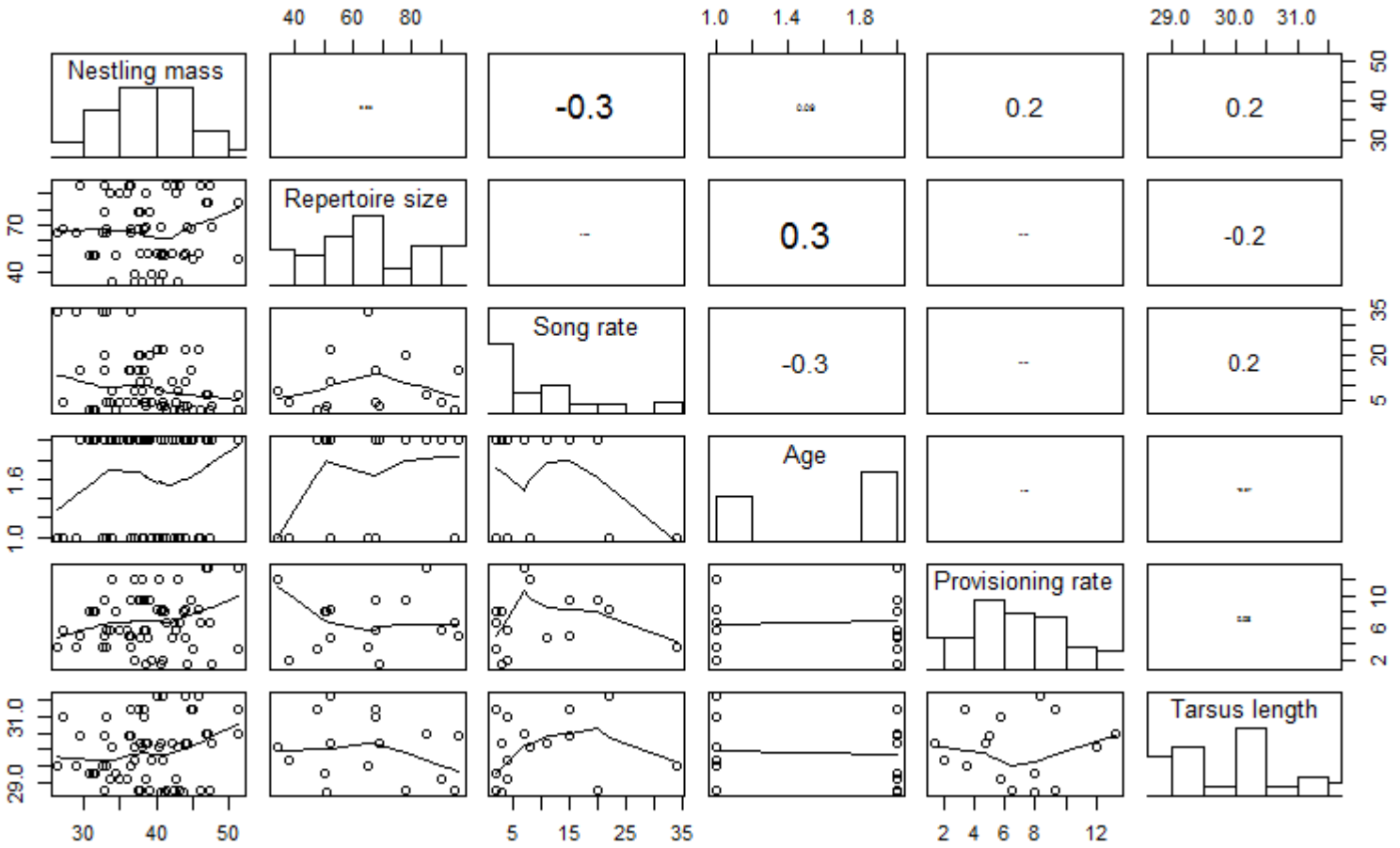


**Figure 4.** Pair plot showing the interactions between all the variables involved in the model investigating nestling mass.

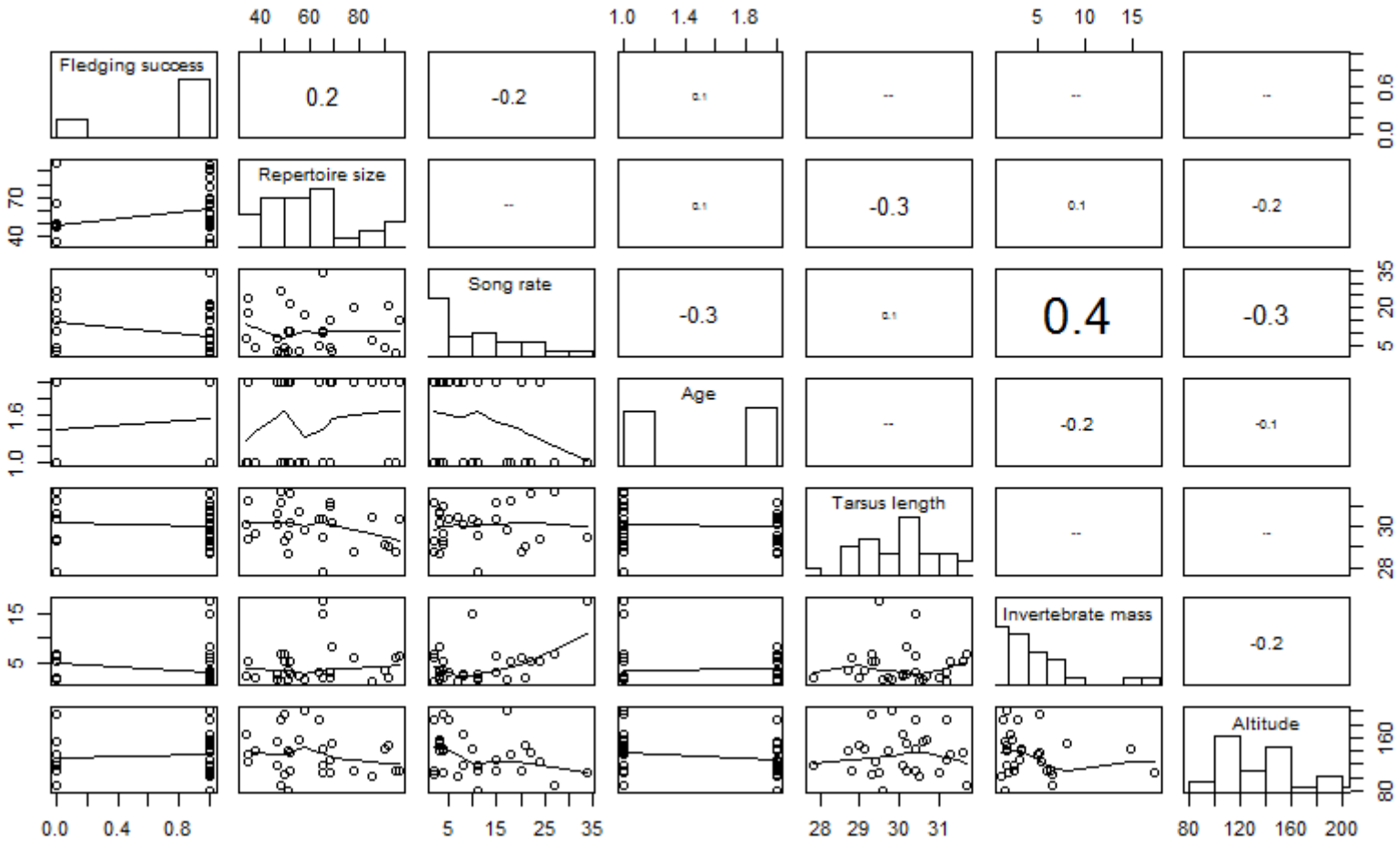


**Figure 5.** Pair plot showing the interactions between all the variables involved in the model investigating fledging success.

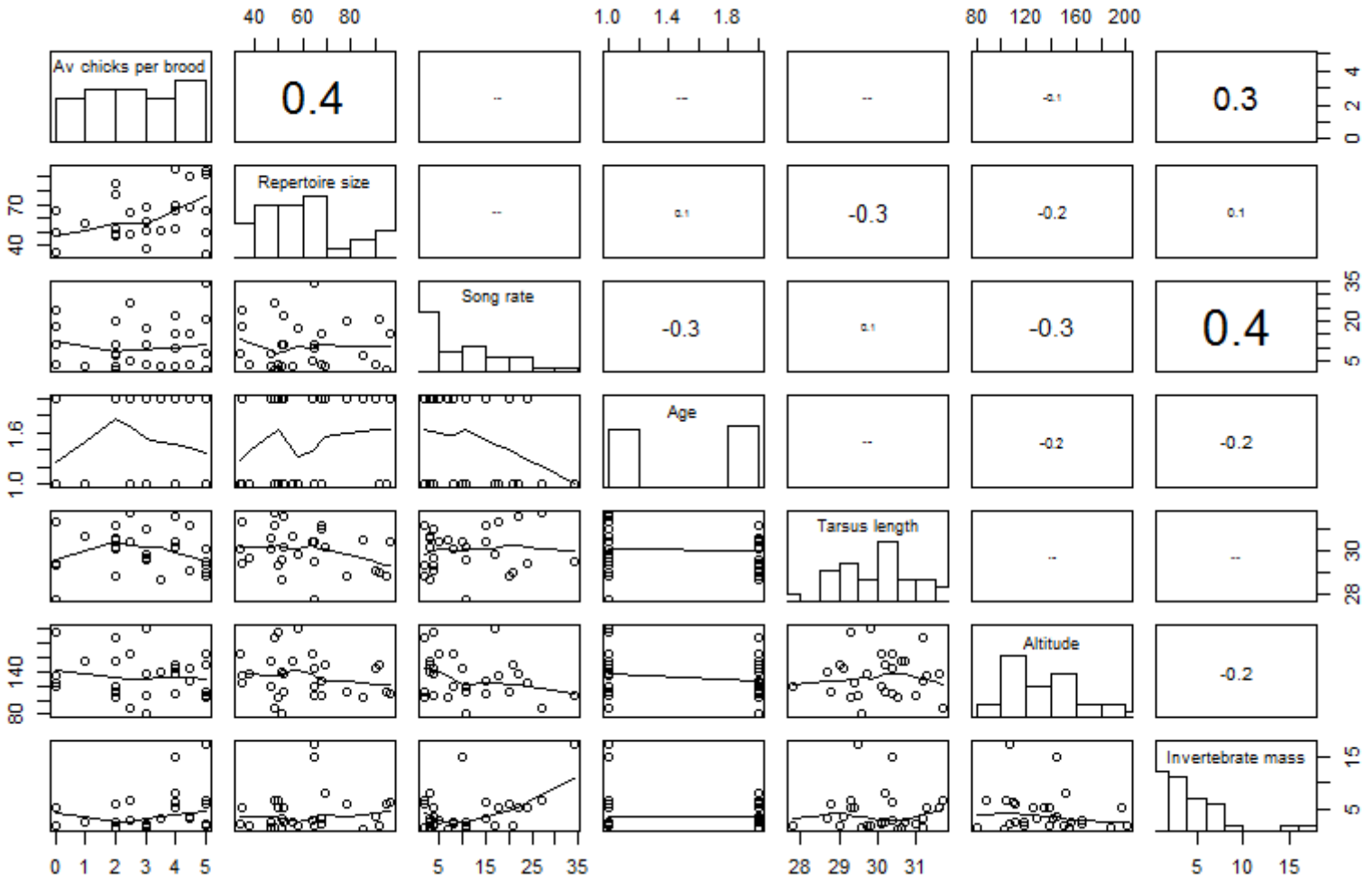


**Figure 6.** Pair plot showing the interactions between all the variables involved in the model investigating the average number of chicks fledged per brood.

**Appendix 4**

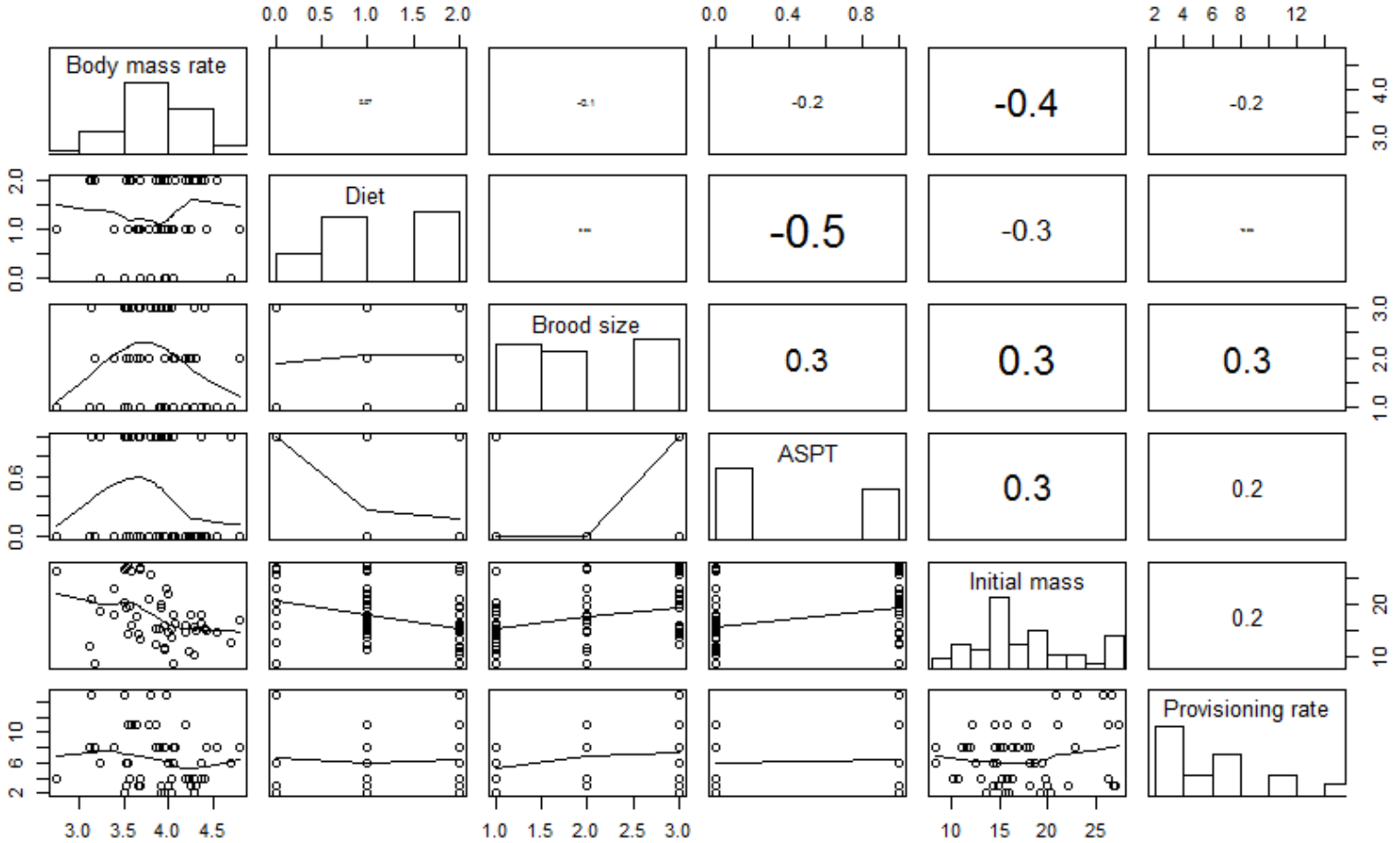


**Figure 1.** Pair plot showing the interactions between all the variables involved in the model investigating body mass growth rate.

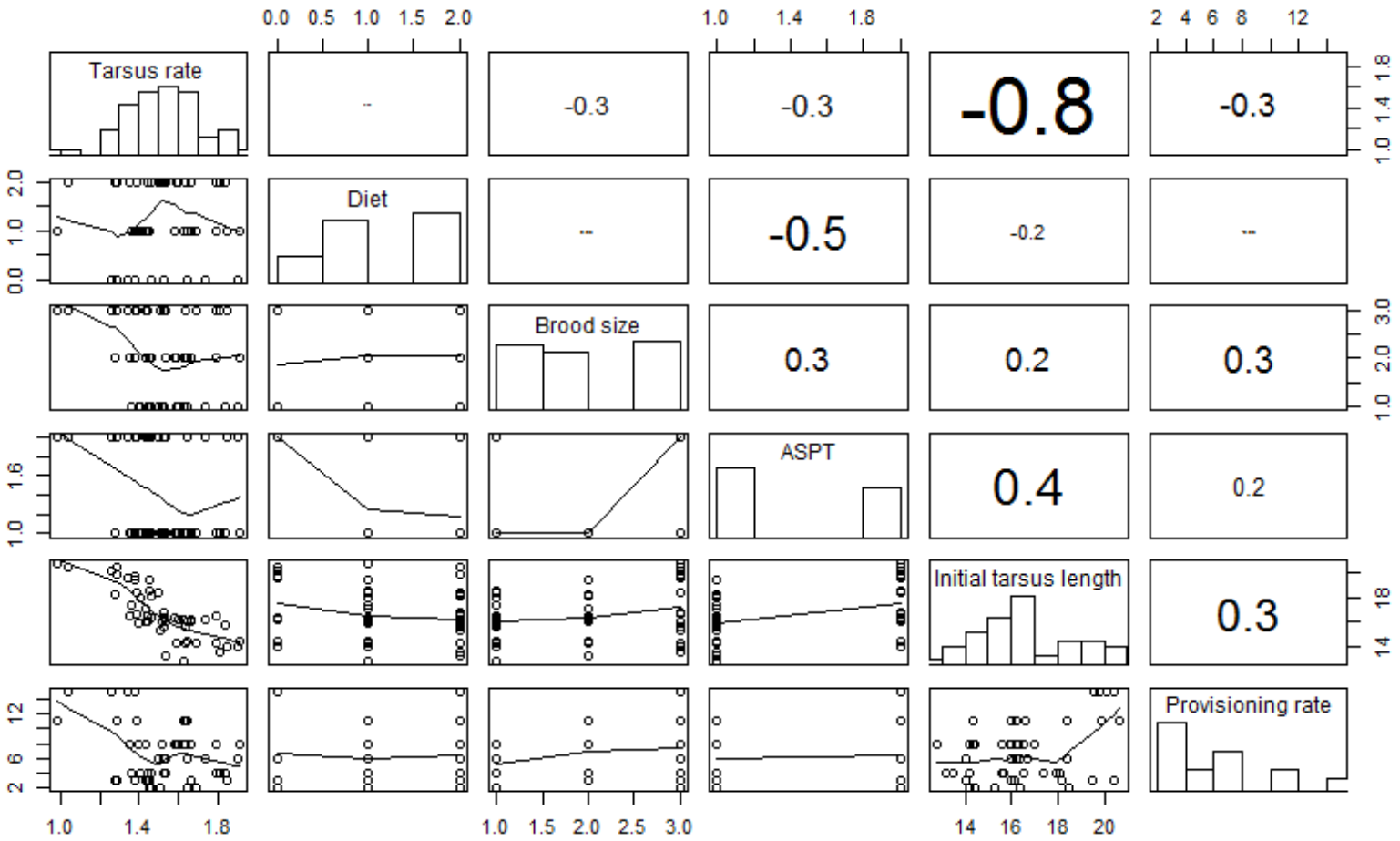


**Figure 2.** Pair plot showing the interactions between all the variables involved in the model investigating tarsus growth rate.

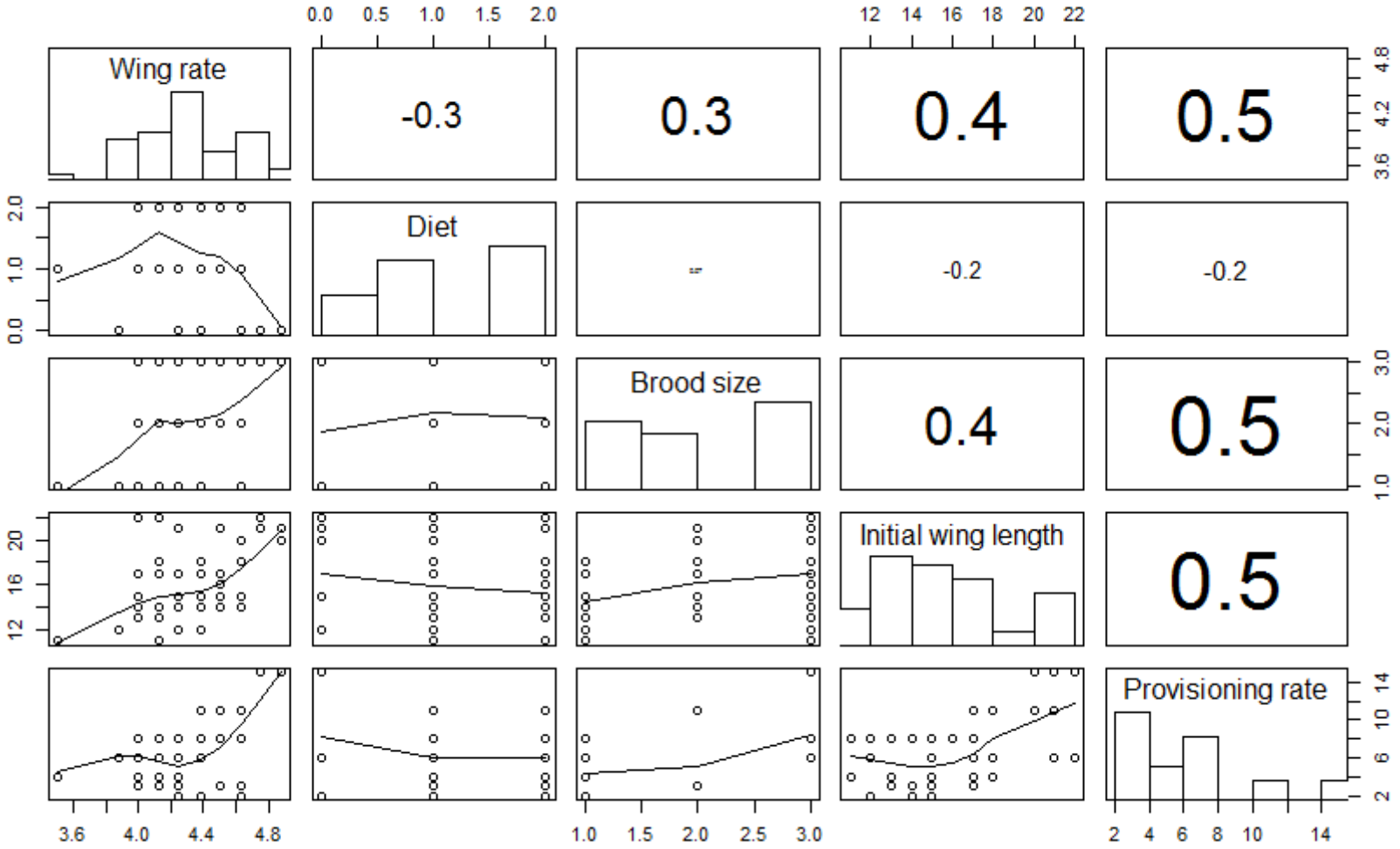


**Figure 3.** Pair plot showing the interactions between all the variables involved in the model investigating wing growth rate.